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Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age

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DOI: <https://doi.org/10.2903/j.efsa.2017.4849>

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ZORA URL: <https://doi.org/10.5167/uzh-144750>

Journal Article

Published Version



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Originally published at:

Hardy, Anthony; Benford, Diane; Halldorsson, Thorhallur; Jeger, Michael John; Knutsen, Helle Katrine; More, Simon; Naegeli, Hanspeter; Noteborn, Hubert; Ockleford, Colin; Ricci, Antonia; Rychen, Guido; Schlatter, Josef R; Silano, Vittorio; Solecki, Roland; Turck, Dominique; Bresson, Jean-Louis; Dusemund, Birgit; Gundert-Remy, Ursula; Kersting, Mathilde; Lambré, Claude; Penninks, André; Tritscher, Angelika; Waalkens-Berendsen, Ine; Woutersen, Ruud; Arcella, Davide; Court Marques, Daniele; Dorne, Jean-Lou; Kass, George E N; Mortensen, Alicja (2017). Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. EFSA Journal, 15(5):e04849.
DOI: <https://doi.org/10.2903/j.efsa.2017.4849>

ADOPTED: 26 April 2017

doi: 10.2903/j.efsa.2017.4849

Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age

EFSA Scientific Committee,

Anthony Hardy, Diane Benford, Thorhallur Halldorsson, Michael John Jeger, Helle Katrine Knutsen, Simon More, Hanspeter Naegeli, Hubert Noteborn, Colin Ockleford, Antonia Ricci, Guido Rychen, Josef R Schlatter, Vittorio Silano, Roland Solecki, Dominique Turck, Jean-Louis Bresson, Birgit Dusemund, Ursula Gundert-Remy, Mathilde Kersting, Claude Lambré, André Penninks, Angelika Tritscher, Ine Waalkens-Berendsen, Ruud Woutersen, Davide Arcella, Daniele Court Marques, Jean-Lou Dorne, George EN Kass and Alicja Mortensen

Abstract

Following a request from the European Commission to EFSA, the EFSA Scientific Committee (SC) prepared a guidance for the risk assessment of substances present in food intended for infants below 16 weeks of age. In its approach to develop this guidance, the EFSA SC took into account, among others, (i) an exposure assessment based on infant formula as the only source of nutrition; (ii) knowledge of organ development in human infants, including the development of the gut, metabolic and excretory capacities, the brain and brain barriers, the immune system, the endocrine and reproductive systems; (iii) the overall toxicological profile of the substance identified through the standard toxicological tests, including critical effects; (iv) the relevance for the human infant of the neonatal experimental animal models used. The EFSA SC notes that during the period from birth up to 16 weeks, infants are expected to be exclusively fed on breast milk and/or infant formula. The EFSA SC views this period as the time where health-based guidance values for the general population do not apply without further considerations. High infant formula consumption per body weight is derived from 95th percentile consumption. The first weeks of life is the time of the highest relative consumption on a body weight basis. Therefore, when performing an exposure assessment, the EFSA SC proposes to use the high consumption value of 260 mL/kg bw per day. A decision tree approach is proposed that enables a risk assessment of substances present in food intended for infants below 16 weeks of age. The additional information needed when testing substances present in food for infants below 16 weeks of age and the approach to be taken for the risk assessment are on a case-by-case basis, depending on whether the substance is added intentionally to food and is systemically available.

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Keywords: infants, neonates, ADI, health-based guidance values, development

Requestor: European Commission

Question number: EFSA-Q-2016-00489

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Acknowledgements: The Panel wishes to thank the members of the Working Group on Substances Present in Food Intended for Infants: Diane Benford, Jean-Louis Bresson, Birgit Dusemund, Ursula Gundert-Remy, Mathilde Kersting, Claude Lambré, Alicja Mortensen (Chair), Colin Ockleford, André Penninks, Josef R. Schlatter, Angelika Tritscher, Dominique Turck, Ine Waalkens-Berendsen and Ruud Woutersen for the preparatory work on this scientific output, and EFSA staff members: Davide Arcella, Daniele Court Marques, Jean-Lou Dorne and George Kass for the support provided to this scientific output.

Suggested citation: EFSA Scientific Committee, Hardy A, Benford D, Halldorsson T, Jeger MJ, Knutsen HK, More S, Naegeli H, Notoleborn H, Ockleford C, Ricci A, Rychen G, Schlatter JR, Silano V, Solecki R, Turck D, Bresson J-L, Dusemund B, Gundert-Remy U, Kersting M, Lambré C, Penninks A, Tritscher A, Waalkens-Berendsen I, Woutersen R, Arcella D, Court Marques D, Dorne J-L, Kass GEN and Mortensen A, 2017. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age. *EFSA Journal* 2017;15(5):4849, 58 pp. <https://doi.org/10.2903/j.efsa.2017.4849>

ISSN: 1831-4732

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Summary

Upon a request by the European Commission to the European Food Safety Authority (EFSA), a guidance was prepared by the EFSA Scientific Committee (SC) for the risk assessment of substances in food intended for infants below 16 weeks of age, taking into particular consideration the procedures to derive health-based guidance values (HBGV).

The EFSA SC considered that for the purpose of this guidance, the term 'infants below the age of 16 weeks' was used to describe the particular infant subpopulations where HBGV had traditionally not been considered applicable. This population includes preterm neonates receiving enteral feeding.

In preparing this guidance, the EFSA SC considered the physiological, developmental and nutritional aspects that are specific for infants below 16 weeks of age, with particular attention to the latest scientific developments in the field.

The EFSA SC concluded that high infant formula consumption per body weight is derived from 95th percentile consumption with the first weeks of life being the time of the highest relative consumption on a body weight basis. Therefore, when performing an exposure assessment for substances in infant formula, the EFSA SC proposes to use the highest consumption figure reported for the period of 14–27 days of life, which corresponds to a value of 260 mL/kg body weight (bw) per day. This value would also cover the potential high consumption rates of preterm infants on enteral (formula) feeding and can be used when carrying out an exposure assessment of substances intentionally or not intentionally added to food, such as contaminants. For the exposure assessment, infant formula is expected to be the only source of nutrition for the first 16 weeks of life in non-breastfed infants.

Gastric, pancreatic and biliary functions are not fully mature at birth. However, preterm and term neonates display a mature-type intestinal epithelium. The local effects of a substance on the gastrointestinal tract, its absorption and its systemic fate will depend on local pH, transit time, size of the absorptive surface or its metabolism by the gastrointestinal wall and by the bacterial microbiome, all parameters changing during post-natal maturation.

Absorption of substances in general might be slower in infants below 16 weeks of age than in older ones, although the amount absorbed is not dependent on the age. Once absorbed and systemically available, the distribution of the substance may be different from that in adults owing to the age-dependent changing of body composition (neonates: larger extracellular and total-body water spaces and lower fat content). Other factors to be considered are the differences in regional blood flow, organ perfusion and cardiac output between infants and adults. In addition, total plasma protein binding capacity (in particular albumin) is lower in infants.

Infants show a unique pattern of development for individual enzyme isoforms involved in phases I and II metabolism, with a reduced capacity at birth. However, owing to overlapping substrate specificities, the overall impact on the clearance and half-life of substances (decrease in clearance, increase in half-life) is limited, being generally up to two- (full-term) to threefold (preterm) lower when comparing infants with healthy adults. Likewise, renal function is also reduced at birth and increases in the first year of life. Therefore, if the precise metabolic pathway is not known and if it is not known to which extent the substance is excreted by metabolic elimination or by renal excretion, an additional uncertainty factor (UF) of 3 should be considered as a conservative option to account for the reduced elimination/excretion. When the precise metabolic pathway for a given substance is known, reported age-specific ratios child/adult can be used. In rare instances where the data are available for the metabolic clearance for this age group, then chemical-specific uncertainty factors can be used. Preterm infants under medical supervision would not be sufficiently protected by the additional UF of 3 due to the considerable immaturity of the absorption, distribution, metabolism and excretion (ADME) system, and would necessitate a case-by-case approach. For compounds, for which effects are depending on long-term body burden (e.g. cadmium, dioxins), the impact of immaturity of metabolism and excretion is likely to be minimal.

The developing blood–brain barrier (BBB) is similar to the adult BBB, in that it possesses functional tight junctions and influx and efflux transporters. Hence, contrary to previous assumptions, the healthy BBB in the first 16 weeks, although immature, is considered functional and not leaky. However, the expression/activity of transporters and metabolising enzymes appears to vary from adult patterns to meet the needs of the developing brain. The movement of molecules across the BBB depends on their physicochemical properties, the local physiological milieu (e.g. pH) and the need for transporters and their ontogeny, activity and specific location. The differences may result in greater vulnerability to chemicals than in the adult. Uncertainty in predicting specific exposure of the central nervous system (CNS) to chemicals derives from knowledge gaps concerning the degree to which alternative pathway

barriers such as the blood choroid plexus barrier are functional and the precise differentiation state of the BBB.

The developing immune system in fetal and early post-natal life is particularly sensitive and exposure to immunotoxicants may result in persistent effects on the immune system that last or appear only long after exposure and in general, and may also occur at lower doses than during adult exposure. In addition, the effects on immune function seen upon perinatal or adult exposure may be different. A number of compounds or types of exposure may produce different ranges or severities of outcomes depending on the time of exposure during the immune system development. Different and unpredictable arrays of alterations (quantitative and qualitative) may be expected when the exposure occurs *in utero* or in the early neonate vs adult exposure. They may be associated with chronic immunological conditions such as autoimmunity, immune deficiency, inflammation and allergic reactions. Altogether, neonates have specific features in the development and maturation of their immune system, which make their response to an immunogenic/allergenic compound different from that of an adult and therefore deserve specific studies.

The development of the female reproductive system in infants below 16 weeks of age is relatively quiescent. However, for the development of the male reproductive organ, the testis, this is a sensitive period. The early post-natal testis exhibits a high proportion of immature Sertoli cells, which proliferate from birth until 6 months of age when the adult number of Sertoli cells is achieved. Effects on the development of the reproductive organs generally may become apparent later in life.

Infants below 16 weeks of age have specific endocrine profiles that are different from those in adults. This suggests that such differences may require particular attention for risk assessment of chemicals, such as those targeting the thyroid. Effects at any early developmental stage may be reflected in deficits at later time points, indicating that the window of exposure, the window of maximum sensitivity and the window of effect require a lifespan approach to generate a fully protective risk assessment.

The EFSA SC notes that, during the period from birth up to 16 weeks, infants are expected to be exclusively fed on breast milk and/or infant formula. The EFSA SC views this period as the time where HBGV for the general population do not apply without further considerations. The EFSA SC made the following considerations when developing a testing strategy for substances in food for infants below 16 weeks of age. Testing strategies for substances present in food for infants need to take into consideration the differences in development stages of the relevant organ systems and the related critical windows of maximum sensitivity between humans and laboratory animals used in safety testing. The safety evaluation of substances that may be present (intentionally or not) in the foods consumed by infants below the age of 16 weeks of age should follow the general principles set for the safety evaluation processes used for older infants and adults and can make use of information already obtained in such studies. The testing programmes for the safety evaluation of substances in food used for the general population will depend on the applicable guidance and legislations. Typically, these studies will provide information on ADME, subchronic and chronic toxicity, genotoxicity, carcinogenicity and reproductive toxicity. However, in view of the potential greater sensitivity during the first few weeks of life, the EFSA SC considers that additional studies may be necessary for the risk assessment of substances present in food consumed by this population.

The EFSA SC has endorsed the following approach to evaluate the safety of substances in food for infants below 16 weeks of age and has developed the following decision tree approach to support a risk assessment.

For substances added intentionally to food for infants below 16 weeks of age, an Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) would be required. The EOGRTS should include cohorts to assess the potential impact of a test substance on the reproductive and developmental system, on the developing nervous system and on the developing immune system. Because exposure through mother's milk in experimental studies is usually substantially lower than the exposure through feed, the resulting doses in the neonatal animals may be insufficient for hazard characterisation. Therefore, for the purpose of this guidance, it is advised that direct dosing of the neonatal animals should be considered as soon as possible after birth. When standard toxicological studies do not show adverse effects in adult animals and the ADME studies show that a substance is not absorbed in relevant amounts, only a repeated dose study with direct oral administration to neonatal animals (e.g. in piglet models) is needed. This latter study should include analysis of possible local effects on the gastrointestinal tract and on a possible reduction in the bioavailability of nutrients (minerals and vitamins) that are normally contained in food for infants. Human data (epidemiological, clinical studies and case reports, where available) should be included in the evaluation. It is possible to

deviate from the recommended testing described in this guidance provided that scientific justification is given. The general principles underpinning the risk assessment performed according to the guidance or recommendations established by the different EFSA panels and units and prevailing legislations should be applied.

For substances not intentionally added to food for infants of this age group, the EFSA SC concludes that their presence in food for infants should be avoided as much as possible. The EFSA SC furthermore considered that current knowledge of the ontogeny of the metabolic and excretory pathways permits now to predict the toxicokinetic behaviour of substances if their metabolism in the adult is known. Even if their metabolic fate is not known in detail, the overall potential impact of the toxicokinetic variability in infants can be addressed by using an additional kinetic UF to account for the differences between the adult population and the infants, in particular the very young ones. However, the EFSA SC is of the opinion that general quantitative and qualitative predictions of adverse effects in infants of this age group cannot be made and that the uncertainty surrounding the toxicodynamic variability in infants of this age group does not allow identification of appropriate additional UFs. Therefore, additional considerations on a case-by-case basis are needed to decide whether there is a health concern. In case studies on neonatal animals are available, these are expected to address the variability discussed above.

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1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

Background

The composition of foods intended for infants and young children is regulated at European Union (EU) level and such rules include requirements concerning the use and/or presence of substances such as food additives, pesticide residues, contaminants and substances migrating from food contact materials in those foods.

Adopting legal provisions on those matters that are in line with the current scientific knowledge requires a comprehensive evaluation to be carried out by the European Food Safety Authority (EFSA) on a number of aspects, including the appropriateness of health-based guidance values (HBGV) for infants below 16 weeks of age.¹

Relevant legal framework

FOOD ADDITIVES IN FOOD FOR INFANTS AND YOUNG CHILDREN

The use of food additives is regulated by Regulation (EC) No 1333/2008² on food additives. Only food additives that are included in the Union list, in particular in Annex II to that regulation, may be placed on the market and used in foods under the conditions of use specified therein. Some of these are specific uses of food additives in food destined for infants and young children (food category 13.1) including uses in food/formulas for infants from birth onwards.

PESTICIDES IN FOOD FOR INFANTS AND YOUNG CHILDREN

Regulation (EU) No 609/2013³ on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control foresees that the specific requirements on pesticides for infant formula, follow-on formula, processed cereal-based food, baby food and food for special medical purposes developed to satisfy the nutritional requirements of infants and young children should be updated regularly and include, *inter alia*, provisions to restrict the use of pesticides as much as possible [Article II (l)(b)].

CONTAMINANTS IN FOOD FOR INFANTS AND YOUNG CHILDREN

The EU rules ensure that food placed on the market is safe to eat and does not contain contaminants at levels which could threaten human health. In order to protect public health, and specifically the vulnerable group of infants and young children, strict maximum levels are established for certain contaminants in foods for infants and young children in the frame of Regulation (EEC) No 315/93⁴.

FOOD CONTACT MATERIALS FOR INFANTS AND YOUNG CHILDREN

Food comes into contact with many materials and articles during its production, processing, storage, preparation and serving, before its eventual consumption. Such materials and articles are called Food Contact Materials (FCMs). FCMs should be sufficiently inert so that their constituents neither adversely affect consumer health nor influence the quality of the food. To ensure the safety of FCMs for consumers including infants and young children, specific migration limits are established for certain substances, for example in the frame of Regulation (EU) No 10/2011⁵.

Terms of Reference

In accordance with Article 29(1)(a) of Regulation (EC) No 178/2002⁶, the European Commission requests EFSA to provide a scientific opinion on scientific and technical guidance for the risk assessment of substances – food additives, pesticide residues, contaminants and substances migrating

¹ Opinion of the Scientific Committee on Food on the applicability of the ADI (Acceptable Daily Intake) for food additives to infants (expressed on 17/9/1998); Opinion of the Scientific Committee for Food on: A maximum residue limit (MRL) of 0.01 mg/kg for pesticides in foods intended for infants and young children (expressed on 19/9/1997) and Further advice on the opinion of the Scientific Committee for Food expressed on the 19 September 1997 on a Maximum Residue Limit (MRL) of 0.01 mg/Kg for pesticides in foods intended for infants and young children (expressed on 4/6/1998).

² OJ L 354, 31.12.2008, p. 16.

³ OJ L 181, 29.6.2013, p. 35.

⁴ OJ L 037, 13.2.1993, p. 1.

⁵ OJ L 12, 15.1.2011, p. 1.

⁶ OJ L 31, 1.2.2002, p. 1.

from food contact materials – present in food intended for infants below 16 weeks of age. In particular, EFSA is requested to provide advice to the European Commission on the generic criteria and/or requirements that need to be considered by EFSA when performing the risk assessment of substances present in food intended for infants below 16 weeks of age taking into account the relevant provisions of the entire above-mentioned legal framework.

1.2. Interpretation of the Terms of Reference

The Terms of Reference require a framework applicable to all relevant working areas of EFSA. Therefore, the EFSA Scientific Committee (SC) will prepare a guidance for the risk assessment of substances in food intended for infants below 16 weeks of age, taking into particular consideration the procedures to derive HBGV.

1. The term 'infants below the age of 16 weeks' is used to describe the particular infant subpopulations where HBGV such as acceptable daily intake (ADI), tolerable daily intake (TDI) or acute reference dose (ARfD) have traditionally not been considered applicable. This follows the earlier conclusions by the former EU Scientific Committee on Food (SCF) and the World Health Organization (WHO) that HBGVs do not apply to populations of infants below the age of 16 and 12 weeks, respectively.

2. The rationale for considering and defining a cut-off value for the age from when a HBGV may apply is complex. It includes different considerations:

- Physiology: maturation of the different physiological processes and organs in the body is completed at different ages, and the time frame may be extremely variable between individuals. Moreover, this population presents a great variability because differences between gestational age (GA) and chronological age may be present (e.g. whether the child is born preterm or at term; the chronological age may not be representative of the actual development).
- Nutrition: diet changes with age after birth, from restricted to milk in the first months of age to a more complex one with the introduction of complementary feeding over time.

The EFSA SC considered that the respective SCF and WHO conclusions appear to be pragmatic rather than scientifically based. Therefore, for the purpose of this guidance, the term 'infants below the age of 16 weeks' is used to describe the particular infant subpopulations where HBGV have traditionally not been considered applicable. This population includes preterm neonates receiving enteral feeding.

3. The guidance should clarify the issues and information requirements that need to be considered when performing a risk assessment of substances in food intended for the population of interest. As specified in the request from the Commission, the Risk Assessment guidance offered is expected to apply to assessing potential risks from food additives, pesticide residues, contaminants and food contact materials. The principles outlined in this guidance may also apply to the risk assessment of other substances which may be added to or are present in food intended for infants below 16 weeks of age.

4. The guidance should consider the physiological and developmental aspects that are specific for infants below 16 weeks of age, with particular attention to the latest scientific developments in the field. The physiological processes and organ systems to be included are the gastrointestinal tract, the metabolic and renal capacities relevant for the elimination of chemicals, the immune-, endocrine- and reproductive systems, and the nervous system including the brain and brain barriers.

5. The guidance focusses on the oral (excluding parenteral) route of exposure because the Terms of Reference relate to substances present in food.

6. The guidance should address specific experimental systems that would be appropriate to characterise the hazard of substances present in food intended for infants below 16 weeks of age.

As regards how to perform an adequate risk assessment for a population of this age, the following points will be discussed in this guidance:

- What makes this population different from the older ones?
- What kind of relevant information can be retrieved from the currently available data from biological experimental studies?
- What kind of data is needed?
- What methodology can be used for risk assessment for this age group? Can a tiered approach defining successive steps of knowledge be applied? Would additional uncertainty/safety factor(s) be required?

1.3. Additional information

The EFSA SC considers that the application of this guidance document is unconditional for EFSA and for all parties submitting assessments of substances present in food intended for infants below 16 weeks of age to EFSA for peer-review (EFSA Scientific Committee, 2015).

2. Data and methodologies

Literature searches were performed in Web of Science (<http://wok.mimas.ac.uk/>) and PubMed (<http://www.ncbi.nlm.nih.gov/pubmed/>) to identify publications in the public domain in the following areas relevant to the developing infant.

- Toxicology
- Nutritional needs
- Physiological and biochemical considerations
 - Physiology of the gut
 - Metabolic and excretory capacities relevant for the elimination of chemicals
 - The nervous system, including brain and brain barriers
 - The immune system
 - The male and female reproductive systems
 - The endocrine system

Comprehensive reviews covering the above areas were identified in order to recognise recent developments since the latest comprehensive assessments of infant toxicology and development by national and international authorities and agencies. The periods covered ranged from January 2012 to February 2016 and the searches were performed in Web of Science. The search limits were document type = review and search terms in title. The search terms combined were 'intestin*' OR 'gastro*' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 98; selected 5); 'metabolism' OR 'cytochrome' OR 'CYP*' OR 'glucuronidation' OR 'glucuronosyl*' OR 'sulfation' OR 'sulphation' OR 'sulfonyl*' OR 'sulphonyl*' OR 'acetylation' OR 'conjugation' OR 'glutathione' OR 'transporter' or 'clearance' OR 'ADME' AND 'development*' OR 'ontogeny' OR 'infant' OR 'neonat*' (retrieved 121; selected 10); 'brain' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 256; selected 21); 'immun*' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 289; selected 14); 'reproduct*' OR 'gonad*' OR 'sexua*' OR 'endocrin*' AND 'development*' OR 'infant' OR 'neonat*' (retrieved 105; selected 25). The selection criteria included coverage of the perinatal or post-natal period and relevance to the assessment. Additional primary references of particular relevance were identified by the working group members (up to May 2016).

In addition, publicly available guidance documents and reports produced by committees and international authorities such as the Joint FAO-WHO Expert Committee on Food Additives (JECFA), Scientific Committee on Food (SCF), EFSA and the European Medicines Agency (EMA) that were relevant to risk assessment of substances in food intended for infants were considered.

A draft of this document Guidance underwent a public consultation from 20 February to 31 March 2017. The comments received were considered and have been incorporated where appropriate.

3. Approach taken to develop this guidance

3.1. Risk assessment of substances present in food intended for infants: past and current approaches

A Food and Agriculture Organization (FAO)/WHO meeting on additives in baby foods was convened in 1971, and its draft report was subsequently modified and adopted by JECFA (FAO/WHO, 1972). The report made an arbitrary distinction between children aged less than 12 weeks and older children, and considered it prudent that food intended for infants under 12 weeks of age should not contain any additives at all. However, if it were deemed necessary to use additives in food intended for young infants, JECFA concluded that 'particularly for infants under 12 weeks, toxicological investigations should be more extensive and include evidence of safety to young animals'. JECFA also concluded that 'the establishment of acceptable residue levels of pesticides or other contaminants likely to be present in milk and cereals for infant foods should be based on toxicological evaluation in very young animals' (FAO/WHO, 1972).

These concepts were further elaborated by the WHO in (IPCS, 1987) where the need for toxicological studies covering the corresponding period of life and the limitation of current testing approaches were noted for cases when young infants are a target population for an additive. This WHO report stated that 'scientific evidence indicates that newborns and very young children are particularly sensitive to the harmful effects of foreign chemicals' due to, *inter alia*, 'immaturity of enzymatic detoxifying mechanisms, incomplete function of excretory organs, low levels of plasma proteins capable of binding toxic chemicals, and incomplete development of physiological barriers such as the blood-brain barrier. Moreover, there appears to be a general vulnerability of rapidly growing tissues, which is particularly important with regard to the developing nervous system' (IPCS, 1987).

In a workshop organised by the ILSI Europe Acceptable Daily Intake Task Force on the applicability of the ADI to infants and children held in 1997, the differences between infants or children and adults from susceptibility and food intake points of view and the adequacy of testing methods and safety factors were discussed (Larsen and Pascal, 1998). Among the recommendations, it was proposed that no special safety factors, and consequently no special ADIs, should be used for infants and children and that any evidence of enhanced sensitivity of this age group to a particular food additive must drive the derivation of the ADI. However, because the usual toxicological test battery does not mimic the human situation with the ingestion of infant formula, the ADI should not be considered directly applicable to infants below 12 weeks of age.

The SCF (1998) expressed an opinion on the applicability of the ADI for food additives to infants. The SCF noted that the scientific support for the 12 week cut-off was not presented in any details in the 1972 JECFA report (FAO/WHO, 1972). While acknowledging the importance of the physiological immaturity of many of the organ systems in the first weeks and hence an enhanced vulnerability, the SCF advocated a 16-week cut-off because of the nutrition habits up to that age (i.e. mother's milk or infant formulae intended for use as the sole source nutrition for infants). Furthermore, the standard animal studies recommended by JECFA and the SCF to assess food additives do not address the exposure situation (i.e. exclusive feeding via infant formula, relevant to this specific age group).

A systematic analysis of the scientific principles to be considered in assessing health risks in children from exposures to environmental agents during distinct stages of development, is reported in (IPCS, 2006). In 2009, the WHO re-iterated its recommendations that 'very young infants are a particularly sensitive subgroup because their metabolic capacities are not yet fully developed' and that 'health-based guidance values are not considered applicable to infants under the age of 12 weeks who might be at risk at lower levels of exposure. Accordingly, risk characterisation of exposure of such infants to chemicals (e.g. in infant formula or occurring as contaminants) has to be considered on a case-by-case basis' (IPCS, 2009).

At its 79th meeting (FAO/WHO, 2014), JECFA evaluated three food additives intended for use in infant formulae and reported general considerations for use of the margin of exposure (MOE) for the evaluation of such food additives. JECFA noted that the ADI concept does not apply to infants up to the age of 12 weeks because they might be at risk at lower levels of exposure compared with older age groups and that risk characterisation for very young infants has to be considered on a case-by-case basis. The report noted that, while reproductive and developmental studies address possible impacts of *in utero* and lactational exposure on neonates, studies involving direct oral administration to neonatal animals are required for the evaluation of additives to be used in infant formulae. If the food additive is intended for use in infant formula at relatively high levels (e.g. 0.1% or greater), then conducting toxicological studies in neonatal animals at doses two or more orders of magnitude greater than the anticipated human exposure, which is the approach commonly taken for food additives, may not be feasible. Therefore, JECFA considered the MOEs between the respective no-observed-adverse-effect level (NOAEL) and the estimated daily exposures to the food additives, and described toxicological and exposure considerations that need to be taken into account in interpreting the MOE (Table 1).

Table 1: Toxicological and exposure considerations to be taken into account in interpreting the margin of exposure (MOE) in the evaluation of food additives used in infant formula, as proposed by JECFA

Considerations related to the toxicological point of departure^(a)
Absorption, distribution, metabolism and excretion in the neonatal organism
Overall toxicological profile of the substance (including identification of critical effects)
Potential effects of exposure during life stages in experimental animals of relevance to human infants
Relevance for the human infant of the neonatal animal models used in toxicological testing
Adverse effects: identified in studies in neonatal animals or is the NOAEL the highest dose tested?
Design and outcome of any clinical studies conducted with infants
Reports of adverse reactions in post-marketing surveillance
Considerations related to the dietary exposure assessments
Formula is the only source of nutrition for the first 12 weeks of life in non-breastfed infants
The additive is used at the maximum proposed level
An energy density of 67 kcal/100 mL (280 kJ/100 mL) is used to convert energy to the volume of formula ingested daily
High infant formula consumption is derived from 95th percentile energy intakes
Variability of exposure among infants is small
Duration of exposure is for a limited time, and exposure decreases on a body weight basis during the exposure period

NOAEL: no-observed-adverse-effect level.

(a): The point of departure (PoD) is equivalent to the reference point (RP) used by EFSA.

3.2. Working definitions for stages in early human development for this guidance

Several classifications of the paediatric population into age categories exist and are to some extent arbitrary due to considerable overlap in developmental (e.g. physical, physiological, cognitive and psychosocial) issues across the age categories. From an EU legislative consideration,⁷ the term 'infant' means a child under the age of 12 months and 'young child' means a child aged between 1 and 3 years. For the purpose of this guidance, the population of interest corresponds to infant subpopulations where health-based guidance values such as ADI have traditionally not been considered applicable, herein referred to as 'infants below 16 weeks of age'. Concerning the stage of physiological development, it is to be noted that this population includes term and preterm neonates (Figure 1).

⁷ REGULATION (EU) No 609/2013 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009.

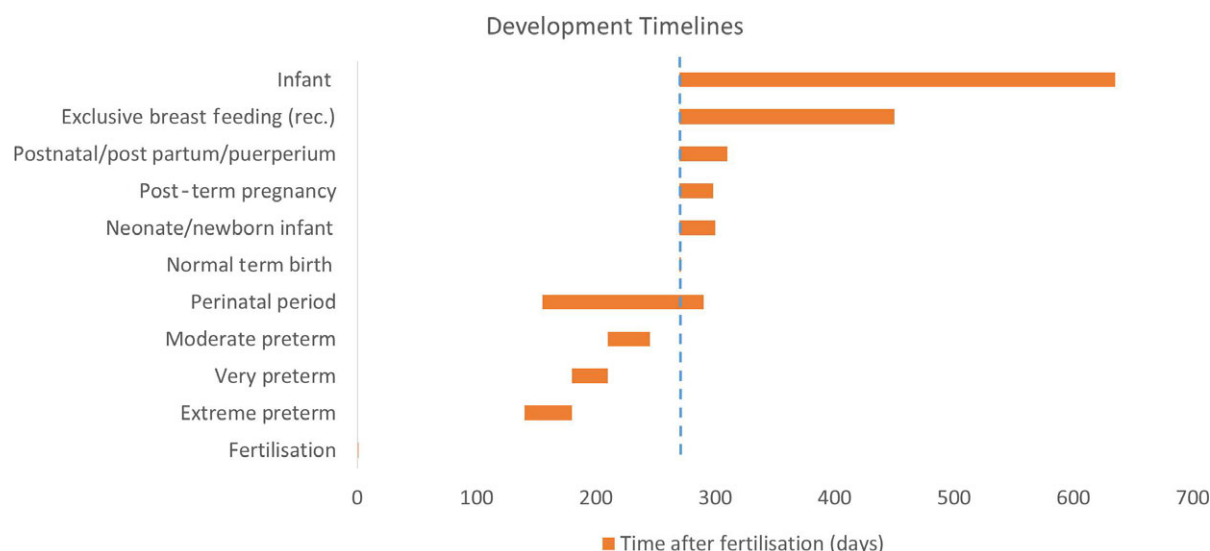


Figure 1: Key life stages

The continuous vertical line represents fertilisation and the dotted vertical line represents normal term birth. Breast feeding may be exclusive or involve infant formula. Abbreviation: rec., recommended.

4. Nutritional needs of infants

4.1. Feeding pattern of term infants during the first months of life

The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) advises that exclusive breastfeeding for around six months is a desirable goal for nutrition of infants (Agostoni et al., 2010). Similarly, the WHO recommends that 'Exclusive breastfeeding for 6 months has many benefits for the infant and mother' and 'Around the age of 6 months, an infant's need for energy and nutrients starts to exceed what is provided by breast milk, and complementary foods are necessary to meet those needs' (WHO, 2001, 2016) (see also Kramer and Kakuma, 2001). If breastfeeding is not possible, infants should receive an infant formula. Regulation (EU) No 609/2013⁸ defines infant formula as 'food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding' [Article 2(2)(c)].

In its Scientific Opinion on the appropriate age for introduction of complementary feeding of infants, EFSA concluded that the introduction of complementary food into the diet of healthy term infants in the EU between the age of 4 and 6 months is safe and does not pose a risk for adverse health effects (EFSA NDA Panel (2009). Therefore, in non-breastfed infants, infant formula should represent the sole source of nutrition until at least the age of four months. The essential composition of infant formula is laid down in EU law. Currently, Commission Directive 2006/141/EC⁹ applies but will be replaced by Commission Delegated Regulation (EU) 2016/127¹⁰, applicable from 22 February 2020, except for infant formula and follow-on formula manufactured from protein hydrolysates, to which it shall apply from 22 February 2021.

⁸ Regulation (EU) No 609/2013 of the European Parliament and of the Council of 12 June 2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control and repealing Council Directive 92/52/EEC, Commission Directives 96/8/EC, 1999/21/EC, 2006/125/EC and 2006/141/EC, Directive 2009/39/EC of the European Parliament and of the Council and Commission Regulations (EC) No 41/2009 and (EC) No 953/2009, OJ L 181, 29.6.2013, p. 35.

⁹ Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC.

¹⁰ Commission Delegated Regulation (EU) 2016/127 of 25 September 2015 supplementing Regulation (EU) No 609/2013 of the European Parliament and of the Council as regards the specific compositional and information requirements for infant formula and follow-on formula and as regards requirements on information relating to infant and young child feeding.

The first months of life are characterised by high requirements of energy intake per kg bw due to the rapid growth rate and increase in body weight (Table 2). While energy intake per kg bw peaks in the first 2 months and decreases afterwards, total energy intake (kcal/day) increases during infancy. Similar changes occur in consumption of breast milk or infant formula when they are the sole source of food for infants in the first 4–6 months of life.

Table 2: Reference values for body weight and energy requirements of infants in the first months of life (taken from EFSA NDA Panel, 2013)

Age	Body weight ^(a)		Average energy requirement		Average energy requirement	
Months	Kg		kcal/kg bw per day		kcal/day	
	Boys	Girls	Boys	Girls	Boys	Girls
0 to < 1	---		109	103	359	329
1 to < 2	4.5	4.2	112	107	505	449
2 to < 3	5.6	5.1	95	92	531	472
3 to < 4	6.4	5.8	78	79	499	459
4 to < 5	7.0	6.4	78	79	546	503

bw: body weight.

(a): 50th percentile of WHO growth standards.

4.2. Consumption data in use by EFSA for the risk assessment of substances present in food for term infants

For infants under 6 months of age, EFSA based its exposure estimates to contaminants present in breast milk and infant formula on standard values for 3-month-old infants using a weight of 6.1 kg, an estimated average daily consumption of 800 mL and a high consumption of 1,200 mL (e.g. EFSA CONTAM Panel (2010)). These figures were derived from the following sources and considerations: 'according to the Institute of Medicine of the U.S. National Academies of Sciences (IOM), average breast milk consumption is about 750–800 g/day (range: 450–1,200 g/day) for the first 4–5 months of life (IOM, 1991). Infant birth weight and nursing frequency have been shown to influence consumption (IOM, 1991). The WHO related breast milk consumption to body weight rather than age with an estimated 125 mL/kg body weight (bw) or 763 mL/day for a 3-month-old child weighing 6.1 kg (Onyango et al., 2002). According to the German DONALD study, mean consumption of infant formula for a 3-month-old child weighing on average 6.1 kg, was 780 mL/day with a 95th percentile consumption of 1,060 mL/day (Kersting et al., 1998). The DARLING study, performed in the USA in the early 1990s, points out significant differences in milk intake between formula-fed infants and breastfed infants: mean milk intake was lower at 3 months (812 ± 133 mL vs 905 ± 162 mL) and 6 months (769 ± 171 mL vs 941 ± 238 mL) in breastfed compared with formula-fed infants, respectively (both $p < 0.05$) (Heinig et al., 1993).

In 2011, EFSA started to use data from the EFSA Comprehensive European Food Consumption Database for the assessment of dietary exposure to hazardous chemicals. This database includes food consumption data for infants up to and including 11 months. In 2011, information on infants were available from only two Member States (Italy and Bulgaria), whereas, in 2015, food consumption data from an additional four Member States (Germany, Finland, Denmark and the UK) were added. Consumption statistics of infant formula (liquid) in infants in the first year of life from the EFSA Comprehensive European Food Consumption Database are reported in Table 3. Food consumption data on infants below 4 months are only available from one survey carried out in Bulgaria, with a 95th percentile of consumption equal to 1,120 mL/day (274.2 g/day per kg bw) for consumers of infant formula. Dietary surveys on infants are currently ongoing in different EU countries within the EU Menu project and their data will be included in the Comprehensive Database as soon as they are made available to EFSA.

At present, data for infants in use by EFSA do not allow time course quantitation of formula consumption during the first 16 weeks of life.

Table 3: Consumption of infant formula (liquid)^(a) in infants from the EFSA Comprehensive European Food Consumption Database

Country	Survey	Age class (months)	Subjects	Consumers (%)	Consumers only			
					Mean		95th percentile	
					mL/day	mL/day per kg bw	mL/day	mL/day per kg bw
Bulgaria	NUTRICHILD	0-3	200	95 (48%)	722	153	1,120	274
		4-6	227	111 (49%)	706	109	1,173	196
		7-12	432	154 (36%)	306	36	700	87
Germany	VELS	6-12	157	33 (21%)	199	24		
Denmark	IAT 2006_07	3-6	38	25 (66%)	420	53		
		7-12	788	519 (66%)	306	34	686	78
Finland	DIPP_2001_2009	6-12	500	293 (59%)	430	54	813	99
United Kingdom	DNSIYC_2011	4-6	76	61 (80%)	637	83	1,029	140
Italy	INRAN_SCAI_2005_06	7-12	1,293	698 (54%)	510	57	854	102
		3-6	3	1 (33%)	625	96		
		7-12	9	1 (11%)	254	25		

bw: body weight.

(a): Infant formula powder transformed into liquid by means of a ratio of 1:8.

4.2.1. Formula consumption over time in healthy non-breastfed term infants during the first 16 weeks

Fomon et al. (1971) reported daily formula consumption in healthy infants throughout the interval of 8–111 days of life (Fomon et al., 1971). The study was carried out between 1966 and 1970, in a sample of 142 Caucasian full-term infants (65 males and 77 females), in a university community in Iowa, USA. Ready-to-feed cow's milk based formula was delivered to the participating families in 120 or 240 mL units to supply for 48–72 h; bottles including unconsumed portions were collected and re-weighed. The energy density of the formula (67 kcal/100 mL) was similar to the energy density of breast milk as reviewed by the EFSA NDA Panel (2014) with 65 kcal/100 g and fits well into the 60–70 kcal/100 g range required for infant formula by the present Commission Directive (Commission Directive 2006/141/EC) and the Commission Delegated Regulation (EU) 2016/127, and as recently proposed by the EFSA NDA Panel (2014).

During days 8–111, daily formula consumption (mL/day) in high consumers (P90) increased up to a maximum of around 1,000 mL/day, which was achieved in boys by the age of 28–41 days and in girls by the age of 84–111 days. Formula consumption related to body weight in high consumers (P90) peaked by the end of the first month at about 200 mL/kg bw (Table 4). Consumption at P95 was not reported for this study sample. However, for a larger sample of this study (380 boys and 340 girls), energy intake per kg bw during the first 8–111 days of life was reported including P95 values (Fomon, 1993). Using the energy density of the formula applied in the study (67 kcal/100 mL), it is possible to recalculate formula consumption as mL/kg bw at P95 in the subsample of the 142 infants from which the original consumption data are available (Fomon et al., 1971) (Table 4). The recalculated P95 consumption values reach a peak by the end of the first month of life of about 220 mL/kg bw in both, boys and girls. It can be assumed that this approach is robust, as the recalculation of the P90 (and P50) formula consumption per kg bw from the reported P90 (P50) energy intake resulted in almost identical consumption values as reported originally for the subsample (Table 4).

Table 4: Consumption of formula in healthy term infants during the first 16 weeks of life

Age (days)	8–13	14–27	28–41	42–55	56–83	84–111
Boys (n = 65)						
mL/day						
P50 ^(a)	573	696	774	790	846	890
P90 ^(a)	741	865	988	1,028	1,033	1,030
mL/kg bw per day						
P50 ^(a)	166	181	172	161	148	137
P90 ^(a)	208	214	209	198	178	155
P95 ^(b)	216	222	213	202	179	164
Girls (n = 77)						
mL/day						
P50 ^(a)	558	657	695	721	741	791
P90 ^(a)	745	757	854	855	909	975
mL/kg bw per day						
P50 ^(a)	160	169	159	153	137	133
P90 ^(a)	208	197	195	182	164	–
P95 ^(b)	213	218	215	198	180	166

bw: body weight.

(a): Daily measurements of liquid formula consumption, 142 infants (65 boys and 77 girls), study period: 1966–1970 (Fomon et al., 1971).

(b): Recalculated from reported energy intake (kcal/kg bw) and a mean energy density of 67 kcal/100 mL formula; energy intake data are from a larger group of infants (boys: n = 380; girls: n = 340) (Fomon, 1993), which included the sample for which original consumption data are presented.

4.2.2. Simulating a consumption time course in healthy non-breastfed term infants during the first 16 weeks with data used by EFSA

While the Fomon data can provide evidence for choosing the 1-month-old infant to reflect high consumption in the first 16 weeks of age, it is not clear whether the data from a study carried out

50 years ago represent current consumption habits. In the long-term observational German DONALD study, 3-day weighed dietary records were collected between 1990 and 1996 in infants aged 3, 6, 9 and 12 months (Kersting et al., 1998). In the diet records, formula consumption was reported as (instant) powder. In the group of consumers of formula ($n = 59$) at the age of 3 months, the P95 value was 24.4 g/kg bw (maximum 24.6 g/kg bw) and a mean \pm SD of 17.1 ± 4.7 g/kg bw, P50 not being reported.

To overcome the problem of different methods used to assess formula consumption in the older study where ready-to-feed bottles were administered (Fomon et al., 1971) and the newer study where powder amount per bottle to be mixed with water was recorded (Kersting et al., 1998), information on today's practice of formula preparation from powder is needed.

Recently, EFSA carried out a search of the Global New Products Database (GNPD) to collect information on preparation instructions on the labels of infant formulae introduced on the European market in the last 24 months (111 products from 13 countries). For most products, one scoop of infant formula powder is to be used for 30 mL of water and the use of the 4.3 g of powder per spoon is the most frequently mentioned scoop size. If this amount (4.3 g) is added to 30 mL of water, it corresponds to 12% (1:8) of the final liquid infant formula. In a recent market search of package instructions of infant formula in Germany (June 2016; eight products, five companies), a similar ratio was found referring to feeding the youngest infants (around 1-month-old).

A recalculation of the potential liquid formula consumption (12%) from the P95 powder consumption 24.4 g/kg bw at the age of 3 months results in a consumption of 195 mL/kg bw (Table 5) which is 18% higher than reported in the Fomon study 50 years ago at the age of around 3 months (Table 4). Starting with this recalculated newer liquid consumption figure at the age of 3 months and using the same course of consumption as in the earlier study, the peak at age 1 month is about 260 mL/kg bw per day. As P50 values of powder consumption (per kg bw) at age 3 months were not reported in the newer study (Kersting et al., 1998), a similar higher consumption by 18% was also assumed for the P50 level and a time course was estimated accordingly (Table 5).

Taking into account all of the available information, the EFSA SC recommends values of 200 and 260 mL/kg bw per day as conservative mean and high level consumption values to be used for performing the risk assessments of substances which do not accumulate in the body present in food intended for infants below 16 weeks of age. These values are derived from data for infants aged 2–4 weeks, when formula consumption is highest, expressed on a body weight basis.

For assessing the exposure to substances with a long half-life and therefore accumulate in the body, it may be appropriate to select consumption values representing a longer period of formula feeding. For example, consumption values for infants of around 2 months of age (56–83 days) (Table 5) could be used, i.e. around 170 (P50) or 210 (P95) mL/kg bw.

Table 5: Estimated consumption of formula using old consumption data during the first 16 weeks of life (Table 4) and newer data on formula consumption at 3 months of age (Kersting et al., 1998)

Age (days)	8–13	14–27	28–41	42–55	56–83	84–111
mL/kg bodyweight per day						
Boys						
P50 ^(a)	196	214	203	190	175	162
P95 ^(a)	254	261	251	238	211	195 ^(b)
Girls						
P50 ^(a)	189	199	188	180	162	157
P95 ^(a)	251	257	253	233	212	195 ^(b)

(a): Estimated as 18% higher than the old data throughout the time course of the first 16 weeks of life based on the 18% higher consumption at P95 in the newer study at the age of 3 months.

(b): As boys and girls were not separated in the newer study, the P95 value at age 3 months was applied for both sexes.

4.2.3. Feeding of preterm infants

The present-day nutrition guidance for preterm infants aims to achieve growth similar to fetal growth coupled with satisfactory functional development (Agostoni et al., 2010).

ESPGHAN (2010) recommends a 'reasonable upper limit' of fluid intake of 200 mL/kg bw (range 135–200 mL/kg bw) for enteral intake of stable-growing preterm infants up to a weight of 1,800 g

(Agostoni et al., 2010). This ESPGHAN recommendation (135–200 mL/kg bw) was confirmed for the 'micropreterm infant' (< 30 weeks gestation, < 10th P birth weight) (Tudehope et al., 2013). For the very low birth weight neonate (< 1,000 g), full enteral feeding with 150–180 mL/kg bw was recommended, and should be achieved by about 2 weeks in < 1,000 g birth weight, and by about 1 week in 1,000–1,500 g birth weight (Dutta et al., 2015).

Because these consumption values are lower than those for term infants in Table 5, application of the high consumption value of 260 mL/kg bw in infants below 16 weeks of age would also cover the potential high consumption rates of preterm infants on enteral (formula) feeding as recommended at present.

5. The developing infant: physiological and biochemical considerations

Most organ and organ systems lack structural or functional maturity at birth. The degree of immaturity may be aggravated due to prematurity, intrauterine growth retardation or any potential pathological condition affecting the infant. Immaturity of physiological processes and organ function predispose neonates to altered toxicokinetics and toxicodynamics, leading to potentially enhanced sensitivity of infants towards substances present in food.

Organ maturation is rapid in the post-natal period, and as a result can yield a high degree of both interindividual and intraindividual variability in the infant population, which might be greater than later in life.

Data are available (ICRP, 2002) from which the relative organ weights can be taken for a newborn compared to the adult. For example, the relative liver weight is 37 g/kg bw in newborns and 25 g/kg bw in adults, the relative weight of the kidneys is 7.1 g/kg bw in newborns and 4.3 g/kg bw in adults. The greatest difference exists for the relative brain weight which is 108 g/kg bw in the newborn and 20 g/kg bw in the adult.

The WHO (IPCS, 2006) reviewed extensively the aspects of organ maturation in infants. These are summarised below, with a particular focus on recent developments in the areas that are pertinent to the risk assessment of substances present in food intended for infants.

5.1. The developing gut

There are reviews available on the intrauterine development of the gastrointestinal tract (Grand et al., 1976; Wells and Spence, 2014). The development is a complex process to which all three germ layers contribute. Among them, and most important, is the endoderm-derived epithelium, which houses specialised intestinal stem cells, that are important for the renewal of the cellular epithelium during the turn-over of the mucosal layer (Sato and Clevers, 2013). Smooth muscle, vasculature and lymphatic are mesoderm-derived, and the nervous system of the gastrointestinal wall has its origin in ectodermal tissue. Besides its role in digestion and absorption of nutrients, the intestine is also a major lymphoid organ through the gut associated lymphoid tissue, which is responsible for the immune tolerance to dietary antigens.

Recently, the colonisation with microbiota of the intestinal tract has become a field of interest also for the post-natal development of the structure and function of the intestine (Di Mauro et al., 2013). However, limited knowledge is available concerning the post-natal development of the human gastrointestinal tract, and whether it is affected by differences in the microbiota.

Local effects in the gastrointestinal tract and absorption and systemic fate of a substance may depend upon its release from the ingested organic matrix, local pH, transit time, size of the absorptive surface or its metabolism by the gastrointestinal wall and also by the bacterial microbiome.

Gastric emptying and intestinal motility

The functional gastric capacity of infants is quite limited. It ranges from 38 to 76 mL in term neonates (Zangen et al., 2001). The coordination of antral contractions is developing after birth, resulting in a marked increase in gastric emptying during the first week of life (Gupta and Brans, 1978). Similarly, increases in the frequency, amplitude, and duration of propagating contractions have been observed to occur in preterm infants (Berseth, 1989; Ittmann et al., 1992). In most studies, the emptying pattern is exponential and can be fitted using the Elashoff model (Elashoff et al., 1982). In preterm infants (24–34 weeks), gastric half-emptying time ($T_{1/2}$) is in the range of 70 min for formula and 35 min for human milk. In full-term infants, data are scarce and $T_{1/2}$ is in the range of 80 min for

formula. Many factors, including caloric density, may affect emptying rate (reviewed by Bourlieu et al., 2014).

The small bowel motility patterns are poorly developed before 28 weeks of gestation, showing a disorganised motility pattern between 27 and 30 weeks, progressing to more mature migrating myoelectric complexes by 33–34 weeks of gestation (Berseth, 1996). Consequently, total transit time is longer in preterm than in term infants. However, interindividual variance is high and sample sizes are small (Bode et al., 2004).

Gastric secretion and digestive enzymes

Gastric acid secretion is active at birth even in very preterm neonates (GA = 24 weeks), and the gastric pH decreases below 4 in the first day of life (Avery et al., 1966). However, the high buffering capacity of human milk and formulae stabilises the gastric pH in the range 5–6; this pH level lasts longer as the meal frequency increases. The pH of gastric content after ingestion of human milk or formulae is optimal for gastric lipase, but not for pepsin activity (Armand et al., 1996). However, extensive proteolysis occurs in the term infant's stomach during the first two weeks of life (Dallas et al., 2014).

The secretion of gastric and pancreatic enzymes is not developed to adult levels at birth. In infants, secretion outputs and enzyme activity levels mature at very different rates: gastric lipase activity already reaches adult levels by 3 months (Menard et al., 1995), whereas pancreatic α -amylase only does so after 3 years of age (Zoppi et al., 1972). Of note, gastric lipase reaches about 75% of adult maximum levels in preterm newborns (Roman et al., 2007). By 1 month post-partum, term and preterm infants' trypsin activity is similar to that of 2–6 years old children (Lebenthal and Lee, 1980). Pancreatic secretion rate of fluids is generally higher in preterm than in term infants. This was also reported for enzyme production in infants of the same post-natal age (Zoppi et al., 1973). Duodenal pH is in the range 5.2–7.6. In newborn infants, bile salt metabolism is active, but immature owing to a not yet fully developed conjugation capacity and slower transport into the intestinal lumen (Poley et al., 1964; Suchy et al., 1981). Therefore, the bile salt pool is limited and the primary bile salt concentration in the duodenum is low (Norman et al., 1972). In contrast, the small intestine of human infants is mature at birth and can handle the various nutrients (Shmerling, 1976). The intestinal surface area is even proportionally greater in infants than in adults because of the rapid prenatal small intestine growth rate (Weaver et al., 1991). Figure 2 provides an overview of the changes in gastrointestinal structure and function with age.

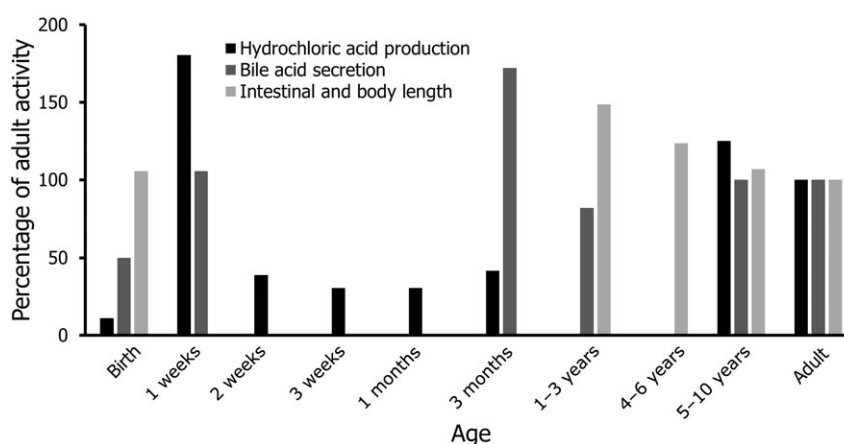


Figure 2: Overview of the age-dependent changes in gastrointestinal structure and function with age. Modified from (Kearns et al., 2003)

The analysis of ileocaecal samples in infants aged 10 days to 5 months and fed 150 mL breastmilk/kg bw per day showed that proteins are almost fully digested and absorbed (Hirata et al., 1965). Balance studies in moderately premature infants (30–36 weeks of gestation) fed either human milk or cow's milk derived formulae have shown that premature infants are also able to digest and absorb proteins (Senterre, 1976). Similarly, the coefficient of absorption of fat in breastfed infants is about 90–95% at 1 week of age and 96% at 1.5 months of age (Williamson et al., 1978). By contrast, fat digestion is less efficient with cow's milk derived formulae as indicated by a 70–85% coefficient of absorption at 3 months of age (Manson et al., 1999). Fat absorption may be even lower in premature

infants (Schmitz, 1977). Despite the slow maturation of pancreatic amylase, most cooked starches are digested and absorbed almost completely as early as 1 month after birth. However, high intakes (40 g/day) induced fermentative diarrhoea in 40% of the infants (DeVizia et al., 1975).

Intestinal permeability

Some animal species (e.g. mouse, rat) retain fetal-type enterocytes at birth. These cells present many cytosolic vacuoles (related to the apical tubular system (ATS)) actively involved in the uptake of luminal material. In mice and rats, fetal-type enterocytes are completely replaced by mature-type ones by the end of weaning, a phenomenon called 'closure'. By contrast, human neonates have mature-type enterocytes at birth, characterised by an adult-type microvillus enzyme pattern (e.g. sucrase) and the absence of vacuoles (Moxey and Trier, 1979; Zabielski et al., 2008). The change from fetal-type to mature-type intestinal epithelium is realised by 18–22 weeks and the ATS is undetectable by 24 weeks of gestation (Milla and Bisset, 1988). Infants born at 34–37 weeks of gestation achieve a 'mature' passive intestinal permeability to lactulose (MW 342; assumed to cross the intestinal wall by a paracellular route) within 4 days after starting oral feeding. 'Mature' intestinal permeability is achieved earlier in exclusively breastfed than in formula-fed infants (Catassi et al., 1995; Taylor et al., 2009). Infants born before 34 weeks of gestation have a higher lactulose permeability than term babies. However, preterm infants show a progressive decrease in lactulose absorption during the first week of oral feeding (Weaver et al., 1984), irrespective of feeding mode (breast milk vs formula) and prebiotic supplementation (Westerbeek et al., 2011).

Gut microbiota

The microbiome in infants is highly dynamic and differs from the microbiome in adults, which is stable and resilient. Schematically, soon after birth, the bacterial microbiome switches rapidly from predominantly facultative anaerobic bacteria towards a diverse community of anaerobes. However, its composition can be notably affected by the mode of delivery (vaginally vs caesarean section), the mode of feeding (breast milk microbiome might be its main physiological template), the environment (from household to geographic location), the host genetics and the use of antibiotics, which may significantly and permanently alter the developing microbiome (Lim et al., 2016). It should be stressed that the reference compositions of the healthy infant and adult microbiota are still unknown.

In the preterm infant, GA, sex and mode of feeding (mother's milk or not), as well as the intensive care unit environment, appear to be the major factors influencing microbiota development (Cong et al., 2016). Schematically, preterm infants display a delay in colonisation with typical commensal bacteria. They show reduced microbiota diversity, reduced levels of strict anaerobes and a high abundance of Proteobacteria.

5.1.1. Summary

Gastric, pancreatic and biliary functions are not fully mature at birth. However, this does not preclude term infants thriving on breastfeeding. It may be particularly relevant to the evaluation of exposure to various chemicals that term neonates display a mature-type intestinal epithelium.

The local effects of a substance on the gastrointestinal tract, its absorption and its systemic fate will depend upon its release from the ingested organic matrix, local pH, transit time, size of the absorptive surface or its metabolism by the gastrointestinal wall and by the bacterial microbiome, all parameters changing during post-natal maturation.

5.2. Ontogeny of the processes involved in the absorption, distribution, metabolism and excretion of chemicals

5.2.1. Absorption

This guidance focuses on oral exposure, and therefore, the term absorption relates to absorption from the gastrointestinal tract.

Only a few studies have been published on the oral absorption of drugs and other substances in infants below 16 weeks of age (Heimann, 1980; Bowles et al., 2010; Batchelor et al., 2014; Batchelor and Marriott, 2015).

In a series of studies, Heimann investigated the absorption of several therapeutically used drugs (sulfonamides, phenobarbital, digoxin and β -methyldigoxin) in paediatric patients in relation to their

age (some days until 1 year) (Heimann, 1980). The amount of absorption, as expressed by the area under the curve (AUC), was not age dependent, whereas the rate of absorption was slower in neonates and increased within the first year to the values observed in adults. Heimann also investigated the absorption of L(+)-arabinose and D(+)-xylose. The amount absorbed of D(+)-xylose, a substance that is taken up through the gastrointestinal wall by active transport, was independent of the age, whereas the rate of absorption was age dependent. The same findings were reported by Heimann for L(+)-arabinose, a substance that is taken up by passive diffusion. Thus, from the findings with drugs and some test substances, it can be anticipated that the absorption of substances in general might be slower in young infants than in older ones but that the amount absorbed is not dependent on the age.

5.2.2. Distribution

Body composition changes with age and hence the physiological spaces to which substances distribute can change. The volume of distribution of a substance, which is not congruent with a physiological space, can change. Factors associated with development such as regional blood flow, organ perfusion, and cardiac output, and also changes in relative organ weights can influence distribution. For example, extracellular and total-body water spaces are larger in neonates and young infants compared to adults (Friis-Hansen, 1983). Also, the adipose stores might be different whereby the percentage of fat is less than 20% in neonates and in young infants and increases in the following months to reach 30% at the age of 9 months. The percentage of extracellular water decreases from 40% in neonates to 30% at 9 months (Figure 3). Details on organ weights and blood flows through the organs in neonates, infants, children, adolescents and adults are given in several sources (ICRP, 2002; Abraham et al., 2005; Mielke and Gundert-Remy, 2009).

As a consequence of the rising body fat, whereas total body water and extracellular water declines, it can be predicted that the concentration of water soluble, polar substances in plasma will become higher and that of fat soluble, non-polar substances in plasma will become lower.

A special case comprises highly plasma protein-bound substances for which altered protein content and binding may have an influence on the kinetics of these substances. The quantity of total plasma proteins (in particular albumin) is reduced during the neonate and young infant period. A higher concentration of bilirubin in neonates might lead to displacement of protein-bound substances from the binding site, thus increasing the non-protein-bound fraction. There are some examples in which this causes an increase in the non-protein-bound fraction of the substance (Ganshorn and Kurz, 1968; Ehrnebo et al., 1971; Fredholm et al., 1975; Windorfer et al., 1978).

The distribution of a xenobiotic can mechanistically be understood as resulting from passive diffusion through membranes. The non-protein-bound, free concentration in plasma is in equilibrium with the free concentration in the tissue (Pelkonen et al., 2008; Smith et al., 2010). Thus, the driving force is the concentration gradients between the binding of the drug in blood and the resulting free concentration and the free concentration in the tissue, which depends on the binding of the drug to tissue components. Depending on the physicochemical properties of the substance and the tissue composition, different tissue to plasma/blood relationship will be established for a substance. Some tissues, also the intestinal wall, express transporters that may transport substances into the cells or out of the cells of the tissue. It is assumed that the activity of the transporters is age-dependent. However, the paucity of experimental data does not allow drawing firm conclusions on this issue (Mooij et al., 2016).

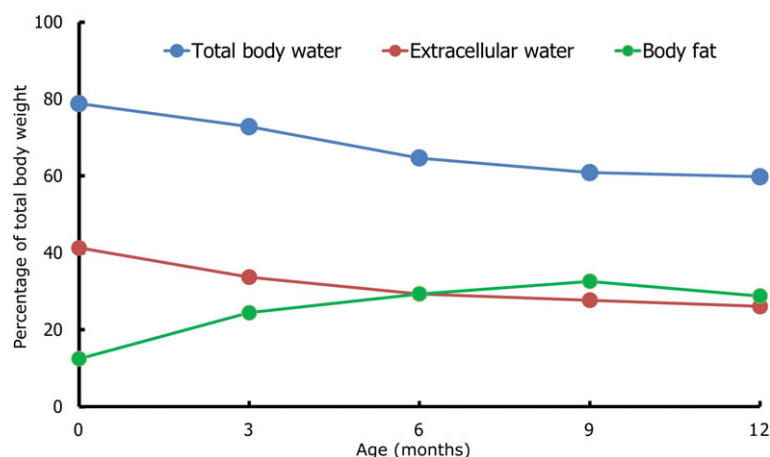


Figure 3: Age-dependent changes in body composition and its influence on the apparent volume of distribution for chemicals. Total-body water and extracellular water, expressed as a percentage of total body weight, is much larger in infants in the first 6 months of life compared to older infants. Modified from (Kearns et al., 2003)

5.2.3. Metabolism and excretion

The main excretory pathways are via metabolism in the liver and renal excretion by the kidney. Volatile agents as well as CO₂, as an ultimate product of metabolism, may be excreted by exhalation. Biliary excretion is for most of the substances a minor excretory pathway in Man. Despite the relatively higher weight of the excretory organs in newborns, their function for the excretion of substances is not yet at the level of the adults with the exception of a few metabolic pathways.

The ontogeny of transporters, xenobiotic drug metabolising enzymes and renal excretion as well as their pharmacokinetic consequences in humans have been reviewed extensively, particularly with the data rich pharmaceutical literature (Renwick et al., 2000; Alcorn and McNamara, 2002; Dorne et al., 2005; Lee et al., 2012; Valcke and Krishnan, 2013; Doring and Petzinger, 2014; O'Hara et al., 2015). Mathematical and pharmacokinetic (PK) models such as physiologically based PK (PB-PK) models have been developed to predict age specific differences, age specific uncertainty factors (UFs) and age- and clearance pathway-specific infant scaling factors (ISFs) (Renwick et al., 2000; Alcorn and McNamara, 2002; Dorne et al., 2005; Valcke and Krishnan, 2013; Salem et al., 2014).

Metabolism

Metabolism involves two large groups of enzymes catalysing phase I and phase II reactions.

Phase I enzymes catalyse key reactions such as oxidation, reduction and dealkylation (e.g. by cytochrome P-450) and hydrolysis (e.g. by epoxide hydrolase and esterases).

Phase II enzymes catalyse conjugation such as glucuronidation by uridine diphosphate (UDP)-glucuronosyltransferase (UGT) isoenzymes (the largest group of phase II enzymes), sulfation by sulfotransferases, glutathione and methyl conjugation by glutathione-S-transferases and methyl-S-transferases, amino acid conjugation (e.g. glycine conjugation) and N-acetylation (NAT-1 and NAT-2). A reduced activity of these enzymes may be responsible for the toxicity syndromes that have been seen in preterm infants, such as grey baby syndrome with chloramphenicol (UGT2B7), neonatal jaundice with bilirubin (UGT1A1) and gasping syndrome with benzyl alcohol (glycine conjugation) (O'Hara et al., 2015).

Discussing the developmental pattern of each enzyme isoform known in humans is beyond the scope of this guidance document.

Generally speaking, each enzyme isoform for phase I and phase II reactions has a unique pattern of development; however, the following three groups have been proposed based on expression profiles in human livers (Hines, 2008; van den Anker et al., 2011; de Wildt et al., 2014) (Figure 4).

- 1) Expression is present during prenatal development with silencing or low level expression within 1–2 years after birth. Specific isoforms include CYP3A7, FMO1, SULT1A3/4, SULT1E1, ADH 1A.

- 2) Expression is at relatively constant levels throughout fetal development and increases post-natally. Specific isoforms include ADH1C, ADH1B, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1, FMO3 and SULT2A1. As an example, the major isoforms of human cytochromes P450 are CYP3A isoforms (CYP3A4/CYP3A5/CYP3A7) which contribute to the metabolism of more than 50% of all marketed drugs and the expression patterns of these isoforms are subjected to developmental influence. As CYP3A7 is highly expressed in fetuses, there is a shift in expression from CYP3A7 to CYP3A4, which occurs within the first month after birth. The shift in expression is associated with a rapid decrease in CYP3A7 after birth, as well as a sharp increase in CYP3A4 in the neonatal period to reach adult levels at one year of age (He et al., 2016).
- 3) Expression with onset occurring mostly in the third trimester with substantial increase in the first 1–2 years after birth. Isoforms include ADH1C, ADH1B, CYP1A1, 1A2, 2A 6, 2A7, 2B6, 2B7, 2C8, 2C9, 2F1, 3A4, FMO3, SULT2A1, UGTs, NAT-2 (Figure 4) (van den Anker et al., 2011).

In humans, total cytochrome P450 content in the fetal liver is between 30% and 60% of that found in the adult and approaches adult values by 10 years of age (van den Anker et al., 2011). CYP1A1 is expressed during early gestation, although expression of most of the other CYP enzymes occurs at or near birth (CYP2B, CYP3A), immediately after birth and reaching adult levels within 30 days (CYP2E1) or after the first week after birth (e.g. CYP1A2, CYP2C). The CYP3A isoform is the most abundant CYP in the human liver (> 50%) of all CYPs and gut and is responsible for the metabolism of more than 50% of known pharmaceuticals and xenobiotics. In terms of isoform, CYP3A7 activity is high immediately after birth; during the first days after birth, there is a shift from mainly CYP3A7 activity to CYP3A4 activity (Lee et al., 2012). Further accounts of the ontogeny of phase I and phase II enzymes are provided in recent reviews (van den Anker et al., 2011; de Wildt et al., 2014; O'Hara et al., 2015).

PB-PK models have been successful at predicting differences between neonates and adults for CYP1A2 using caffeine and theophylline and CYP3A isoforms but poorer predictions were obtained for CYP2D6 and CYP2C because of lack of model complexity or inadequate hepatic microsomal activity data to fully describe the maturational process of functional enzymes (Alcorn and McNamara, 2002; Ginsberg et al., 2004). Recently, several studies reported clearance values in neonates predicted using *in silico* tools that incorporate *in vitro* enzyme PK parameters (maximum rate of metabolism (V_{max}) and Michaelis–Menten constant (K_m)), *in vivo* clearance data from the literature in neonates and ontogenic information of isoforms. In 70% of cases, the predicted median clearances in neonates were within two-fold of the observed values (Johnson et al., 2006; Salem et al., 2014).

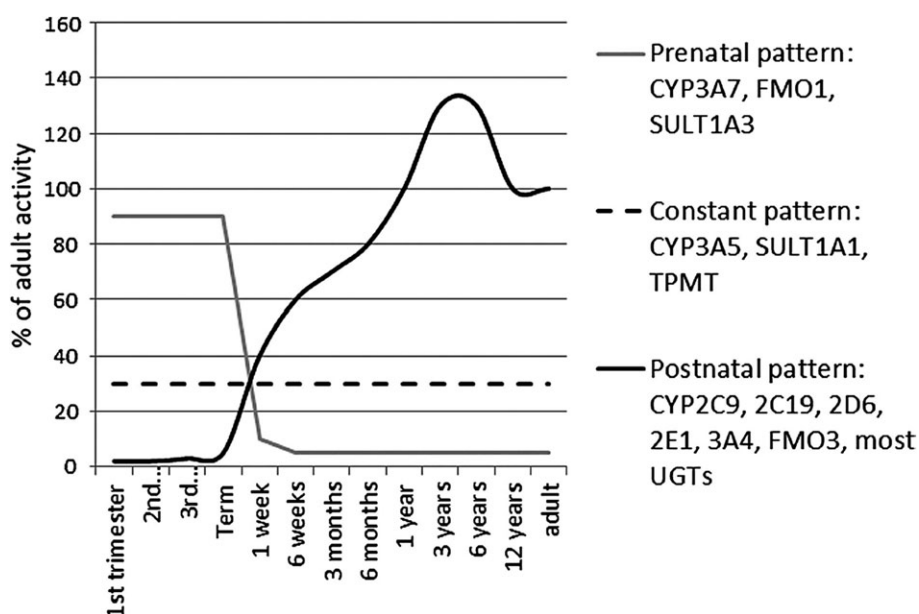


Figure 4: Schematic depiction of drug-metabolising enzyme ontogeny. The post-natal pattern is expressed as percentage of the adult level. Abbreviations: CYP, cytochrome P450; TPMT, thiopurine S-methyltransferase; UGT, UDP-glucuronosyltransferases; SULT, sulfotransferases; FMO, flavin-containing mono-oxygenase. Reproduced with permission (de Wildt et al., 2014)

Finally, the extent of metabolism can be influenced by a number of factors including polymorphic expression of enzymes and transporters (CYP2D6, CYP2C9, CYP2C19, OATP, P-Gp) and the ontogeny of the rate of hepatic blood flow (particularly relevant for compounds with a high hepatic ratio). Immediately after birth, blood flow through the liver rapidly changes with the ductus venosus closing. In most cases, full closure will be within 1 week. With the first feed, portal blood flow increases leading to a rapid increase in hepatic functions and induction of a number of enzyme groups. With feeding bacterial colonisation of the gut begins, and varies with the type of feeding (i.e. breastfeeding or formula). Several studies have noted that because of such acute changes standard pharmacological models of hepatic extraction, metabolism and blood flow are not applicable to neonates and that they are relevant only once the ductus venosus is fully closed (van den Anker et al., 2011).

Excretion

Excretion is a key step in ADME processes being the final removal of a xenobiotic and/or its metabolites from the body mostly via the renal (i.e. urine) or hepatic routes (i.e. faeces). Renal excretion is particularly important for drugs and xenobiotics that are excreted as parent compound without metabolism and water-soluble metabolites if they possess toxicological properties (Mooij et al., 2016). When dealing with renal excretion of chemicals, renal clearance can be several-fold lower in neonates compared to older children and adults for lipophilic, hydrophilic, and organic ions (van den Anker et al., 1995; Clewell et al., 2002). Creatinine is a standard marker of renal function that becomes an accurate biomarker during the first weeks of post-natal life (O'Hara et al., 2015).

PB-PK models in neonates and young infants for drugs and environmental chemicals that are primarily cleared by the kidney have been developed using data for rates of glomerular filtration, tubular secretion, tubular reabsorption, and renal blood flow (Alcorn and McNamara, 2002; Johnson et al., 2006; DeWoskin and Thompson, 2008; Salem et al., 2014).

The maturation of the kidney, or nephrogenesis, is complete within 36 weeks of gestation followed by post-natal changes in renal and intrarenal blood flow. The glomerular filtration rate (GFR) at birth is approximately 2–4 mL/min per kg in term neonates (one third of the adult value expressed on the basis of body surface area) and as low as 0.6–0.8 mL/min per kg in preterm neonates. During the first 2 weeks of life, GFR increases rapidly to half of the adult value within 3 months and then follows a steep rise to adult values within 8–12 months. Such an increase in GFR in the first weeks of life is mainly driven by an increase in renal blood flow. Tubular secretion is around 25% of adult levels at birth, and increases more slowly and more variably than GFR, not approaching adult levels until 1–5 years of age. Limited data on renal plasma flow indicate neonatal rates of only 10–20% of adult values that rapidly increase to 50% by 6 months, and then approach adult levels by 1–2 years of age (Alcorn and McNamara, 2002; van den Anker et al., 2011).

Kidney maturation in the neonate also involves changes in the expression of a number of transporter isoforms together with developmental changes in signal transduction affecting the capacity of renal tubules to reabsorb water and solutes. However, beyond expression levels, little is known about the ontogeny of human membrane transporters and its consequences on the PK of xenobiotics (Brouwer et al., 2015; Gattineni and Baum, 2015).

The renal function reaches 50% of the activity of adults within the first 2 months of age. A number of analyses showed overall 1.5- to 3-fold differences between neonates, infants and healthy adults for renal clearances and total clearances and half-lives of substances that are cleared by the kidney (e.g. antibiotics) (Hattis et al., 2003; Dorne et al., 2005; Valcke and Krishnan, 2013) (Figure 5). For the oral route, limited data showed that clearances were slightly higher in neonates and infants compared with healthy adults and associated with larger variability. For renal excretion, it is considered that an additional UF of 2–3 would cover the differences between adults and infants below the age of 16 weeks. However, so far, the analyses from the literature have been based on compounds that are eliminated rapidly by the kidney and comparative elimination patterns between neonates, infants and healthy adults and for persistent compounds are as yet unavailable.

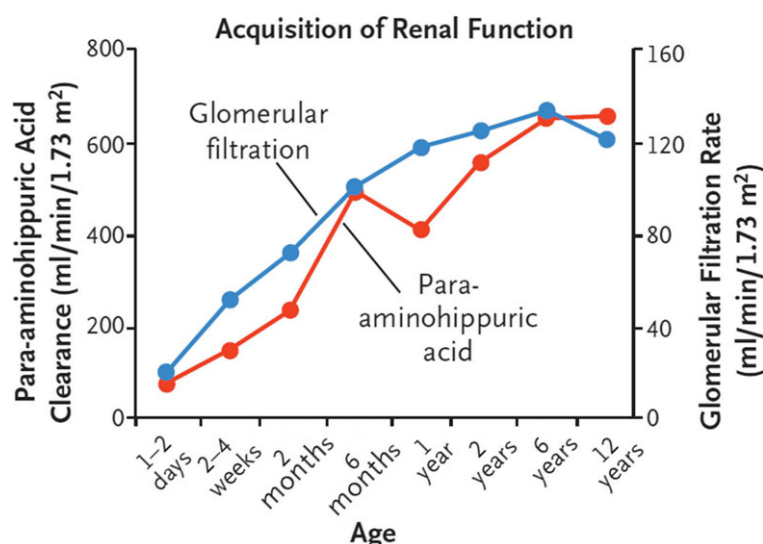


Figure 5: Maturation of renal function in the infants and young children. The figure shows para-aminohippuric acid clearance, reflecting the ontogeny of renal blood flow, and glomerular filtration rate as a function of age. Please note the non-linearity of the scale for the X-axis. Reproduced with permission (Kearns et al., 2003)

To illustrate the influence of immature renal function and immature enzyme activity, the example of the kinetics of propylene glycol should be mentioned. Propylene glycol is both eliminated by metabolism through alcohol dehydrogenase and by renal excretion. In adults, renal excretion accounts for 45% of the clearance (De Cock et al., 2014). In a study by de Cock et al. (2013), infants with GA between 24 and 41 weeks at birth, were investigated at an age between 1 and 82 days and a birth weight between 630 and 3,980 g (De Cock et al., 2013). The mean clearance in this infant population was 0.085 L/h whereas the clearance in adults is known from the literature to be 8.64–23.4 L/h per 1.73 m² (Speth et al., 1987). The clearance of propylene glycol was dependent on post-natal age and on body weight of the infants. This example also shows that this guidance cannot give general recommendations for preterm newborns and that HBGVs for substances used in this age group require special consideration.

Transporters

Membrane transporters can play an essential role in the transport of endogenous and exogenous compounds, and mediate the uptake, distribution, and excretion of many drugs. Historically, such transporters were not considered with phase I and phase II metabolism and, over the past two decades, phase I and II metabolism has been extended to a concept of four phases, which now includes phase 0, namely the cellular uptake of compounds, and phase III, the transport of compounds out of the cells. Cellular transporters belong to two main clusters of transporter families: the solute carrier (SLC) families and the adenosine triphosphate (ATP) binding cassette (ABC) carriers. SLC transporters include the human organic anion transporting polypeptides (OATPs) and human organic cation transporters (OCTs), and are often denominated as phase 0 uptake transporters. ABC transporters are often called efflux pumps or phase III and include examples of importance such as P-glycoproteins and multidrug resistance proteins (MRPs) (Hillgren et al., 2013; Doring and Petzinger, 2014; EFSA, 2014). Transporters are mostly expressed in liver, kidney, the adrenal gland and the lung (Doring and Petzinger, 2014).

Examples of transporters of physiological importance in the kidney include the OATPs expressed in the kidney that are involved in the final excretion of phase II conjugates. OATPs have low activity at birth then increase rapidly to high levels, higher than those seen in adults, over the first few weeks of life and then begin to decline to adult levels. Such a change in secretion is independent of the changes in renal mass. Exposure to hormones and antibiotics, such as penicillin, also increases organic anion transporter activity (Mooij et al., 2016).

Ontogeny of metabolic pathways and its impact on clearance

Ontogeny of metabolic pathways in neonates has been shown to be isoform-specific and the quantification of its impact on acute and chronic elimination of xenobiotics is an important aspect of risk assessment (Hattis et al., 2003; Dorne et al., 2005; Valcke and Krishnan, 2013). If the enzymes involved in the metabolism of the substance are known, the expression level of the enzymes can be taken into consideration. For example, newborns need only 25% of the dose of a one-year-old infant to get the same plasma concentration of morphine, which is metabolised by UGT2B7 and other UGT isoforms (Knibbe et al., 2009; Sato et al., 2012).

If the enzymes involved in the metabolism of a substance are not known, it should be assumed that the activity levels of the enzymes are lower compared to adults. Consequently, the clearance of substances may be lower compared with the adult population. Since the clearance is the product of the elimination constant ($\ln 2/\text{half-life}$) and the volume of distribution, both age-dependent in infants, half-life and clearance are not linearly related. The pattern is illustrated in Figure 6 from Valcke and Krishnan (2013) based on the data of (Ginsberg et al., 2002) and (Hattis et al., 2003).

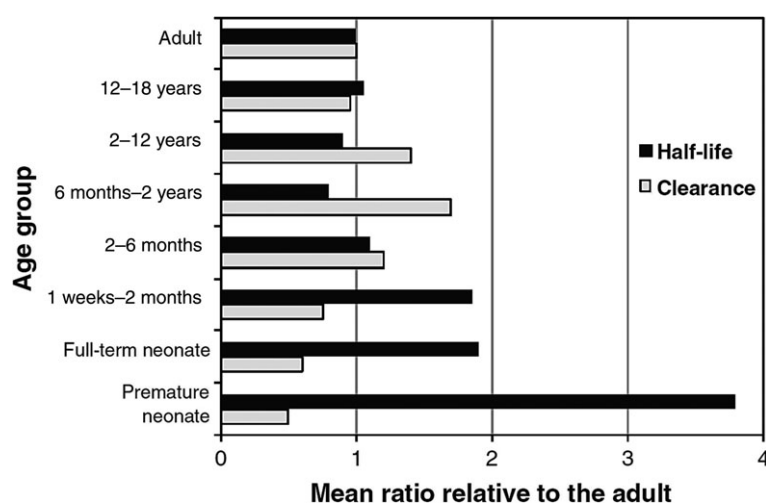


Figure 6: Half-life and clearance ratios (child/adult) as a factor of age. Reproduced with permission Valcke and Krishnan (2013)

Other studies have given more detailed information, indicating the relative activity of specified enzymes in children of different ages versus the adult (Table 6).

Table 6: Estimates of age-dependent enzyme activity as ratio child/adult

Age	Enzyme activity (ratio child/adult)				
	CYP 3A4	CYP 1A2	CYP 2E1	UGT 2B7	UGT 1A6
Premature	0.1	0.02	0.1	0.015	0.015
Term	0.2	0.05	0.21	0.05	0.1
7 days	0.24	0.1	0.32	0.06	0.11
1 month	0.5	0.2	2.5	0.1	0.16
3 months	0.7	0.25	0.45	0.3	0.25
6 months	1.1	0.29	0.45	0.7	0.36
1 year	1.25 (1-3 years)	0.34	1.0	1.0	0.5
10 years	1	1 (8 years)	1	1	1

Modified from (Edginton et al., 2006). Values similar to those given by Edginton et al. (2006) have also been reported in other studies (Cresteil et al., 1985; de Wildt, 2011; Saghir et al., 2012).

CYP: cytochrome P 450; UGT: uridine diphosphate glucuronosyltransferase.

It should be noted that in contrast to other conjugation enzymes, sulfotransferases are expressed at the same level in infants as in young adults as indicated by semi-quantitative Western blot analyses of liver samples (Duanmu et al., 2006).

As a generalisation, if the substance is mainly metabolised, the reduced metabolic activity of most of the enzymes will lead to higher concentration in plasma. If the toxicity is owing to the parent compound, the substance may have a higher toxicity owing to the higher internal exposure. A more complex situation is present if the toxicity is due to bioactivation of the parent compound. The resulting toxicity depends on the potential presence of parallel metabolic pathways with a more advanced development leading to non-toxic metabolites by which the parent compound will then mainly be eliminated and/or on the maturity of the kidneys, which may excrete the parent compound and the formed metabolites being toxic or non-toxic.

If the enzymes involved in the metabolism are not known, the general approach by Valcke and Krishnan (2013) can be applied. The study provided half-life and clearances for different age groups as the ratio child to adult based on the data of Ginsberg et al. (2002) and Hattis et al. (2003) that analysed kinetic studies, mainly of drugs, in the different age groups. The data given in Figure 6 (Valcke and Krishnan, 2013) are the means of the ratios from all the kinetic studies. If the metabolic pathway and the involved enzymes of a substance are known, available detailed information could be used to adjust for enzyme activity in the specific age group. The activity of several CYP-enzymes and of other enzyme can be taken from Table 6, which is based on a publication by Edginton et al. (2006).

5.2.4. Summary

Absorption of substances in general might be slower in infants below 16 weeks of age than in older ones, but the amount absorbed is not dependent on the age. Once absorbed and systemically available, the distribution of the substance may be different from that in adults owing to the age-dependent changing of body composition (neonates: larger extracellular and total-body water spaces and lower fat content). Other factors to be considered are the differences in regional blood flow, organ perfusion and cardiac output between infants and adults. In addition, the total plasma protein binding capacity (especially albumin) is lower in infants.

Infants show a unique pattern of development for individual enzyme isoforms involved in phases I and II metabolism, with a reduced capacity at birth. However, owing to overlapping substrate specificities, the overall impact on the clearance and half-life of substances (decrease in clearance, increase in half-life) is limited, being generally up to two- (full-term) to threefold (preterm) lower when comparing infants with healthy adults. Likewise, renal function is also reduced at birth and its function increases in the first year of life. Therefore, if the precise metabolic pathway is not known and it is not known to which extent the substance is excreted by metabolic elimination or by renal excretion, an additional UF of 3 could be considered as a conservative option to account for the reduced elimination/excretion. When the precise metabolic pathway for a given substance is known, the age-specific ratio child/adult (Table 6) can be used. In rare instances where the data are available for the metabolic clearance for this age group, then chemical-specific UFs can be used. Special considerations may need to be taken into account if a substance is metabolised through a pathway that is polymorphic, such as for CYP2D6 substrates. The importance of the polymorphic expression of, for example, CYP2D6 depends on the relative contribution of this metabolic pathway to the overall elimination. A case-by-case approach is advisable to account for a potential higher susceptibility of poor metabolisers in all age groups.

Specific situations may exist that will affect the impact of the immaturity of the infants ADME system on the magnitude of the difference in internal dose between infants below 16 weeks of age and adults and the potential toxicological consequences. For example, extremely and very preterm neonates represent a special subpopulation under medical supervision that would not be sufficiently protected by the additional UF of 3 due to the considerable immaturity of the ADME system, and would necessitate a case-by-case approach.

For compounds for which effects are dependent on long-term body burden (e.g. cadmium, dioxins), the impact of immaturity of metabolism and excretion is likely to be minimal.

5.3. The developing brain and brain barriers

When describing the development of the central nervous system (CNS), the differential role between the developing brain barriers (BBs) and the developing neuronal and glial cells needed considering. In the context of chemical risk assessment, a key impact of the BB is on the accessibility of compounds to their target neuronal and glial cells, and as such, the BB can be regarded as a major toxicokinetic modulator for the distribution of chemicals and other substances. The BB influences the distribution to the target cells and hence the concentration at the site of action, whereas the ability of

a chemical to induce adverse effects on neuronal or glial cells reflects the toxicodynamic properties of the compound.

5.3.1. The developing brain barriers

Once a potential neurotoxin has entered the systemic circulation, it will reach the CNS via cerebral blood vessels. The blood–brain barrier (BBB) is formed by endothelial cells, which, in contrast to other endothelial cells, have continuous intercellular tight junctions, lack fenestrations and undergo extremely low rates of transcytosis. Astrocytes, pericytes and extracellular matrix components contribute structurally and functionally to the BBB (Obermeier et al., 2013). However, the brain and spinal cord are bathed in fluid that is chemically different from the blood (Saunders et al., 2014). In a risk assessment, it is therefore desirable to understand the BBs and how they control access of substances, to the neuronal cells of the CNS at all stages of development.

Development of the BBB commences as the neural tube forms and mesenchymal cells surround and penetrate developing neuroectoderm. Because the brain grows and new blood vessels form throughout gestation and well into the post-natal period, the barrier is continually forming fresh new areas and expanding. The barrier is probably functional from very early in the development of the CNS (Saunders et al., 2014). The key component of the BBB is a continuous vascular endothelium controlling bidirectional transport of large and small molecules through regulation of transcellular and intercellular pathways between circulating blood and the neuronal compartment.

The observation of unconjugated bilirubin penetration to deep human brain structures in the condition of neonatal kernicterus was previously taken incorrectly as evidence for the BBB being leaky in the period shortly after birth (Goldmann, 1913; Grøntoft, 1954; Kumar et al., 2010). However, it is now generally accepted, based on considerable experimental evidence from vital dye and radioactive tracer experiments, that this is not correct and that the barrier in the fetus and newborn should be described as 'immature' and not as 'leaky' (Saunders et al., 2014).

Extent of the BBBs

There are several (albeit some relatively minor) routes that allow access to the CNS and brain that lie parallel to the BBB. One is the route to the hindbrain vomiting centre where substances derived from blood gain direct CNS access allowing them to create part of a neurochemical reflex that is protective against ingested poisons.

In dye injection studies in humans and animals, performed within hours of birth, the brain was generally unstained although dye did stain smaller specific parts including the choroid plexus and the circumventricular organs, which lie just outside the BBB. The substantially higher concentration of protein in cerebrospinal (CSF) observed at the time of maximum growth of the brain is caused by specific transport from the blood to the CSF across the choroid plexus epithelium. Therefore, this region has been called the blood–CSF barrier (Adinolfi et al., 1976; Adinolfi, 1985; Liddel et al., 2011a, 2011b, 2013). A blood–retinal barrier is also considered as a distinct interface, as are the roof of the third and fourth ventricles, capillaries in the pineal gland, the roof of the diencephalon and the pituitary gland where there is release of endocrine secretions directly into the blood stream. Uncertainty stems from the fact that study of transport at several of these sites has not been extensive.

Permeability and transport of substances across the BBB

Substances can gain access to the glial and neuronal cells by simple passive diffusion across the BBB (which physicochemically can be regarded as an interrupted phospholipid membrane) or by facilitated diffusion or by active transport, depending on their physicochemical properties. Lipophilic substances will cross the BBB by passive diffusion, whereas, as in the case of water-soluble compounds, specific membrane transport proteins play a major role for their transport across the BBB and in and out of the cells (Obermeier et al., 2013). Small molecules such as amino acids and glucose are transmitted across endothelial cell plasma membranes by transporters, carriers and channels. Brain endothelia exhibit ion channels affecting Ca^{2+} flux and vasoactive secretion of nitric oxide, histamine, substance P, endothelin-1, bradykinin also prostacyclin, haemostatic factors such as tPA and von Willebrand factor (Ennis et al., 1996; Kimelberg, 2004). Macromolecules appear to use clathrin (cationic preference) and caveolin (anionic preference). These coated endocytic and transcytotic pathways may lead to lysosomal degradation of their contents or may carry their contents intact

across the endothelium (Narushima et al., 1999; Lossinsky and Shivers, 2004). Transport across the BBB can be paracellular through the intercellular space between the cells or transcellular.

Paracellular transport across the BBB is by passive or facilitated diffusion alone. It is based on electrochemical, hydrostatic and osmotic gradients, characterised by higher conductance and lower selectivity, has similar conductance and selectivity in either apical-to-basal or basal-to-apical directions and has well defined values of electrical conductance, as well as charge and size selectivity.

Evidence that endothelial gene expression patterns differ in development, observed higher rates of synthesis of certain proteins at early time points and differential expression of membrane transporter proteins can account for the majority of the differences in solute composition of the CNS interstitial fluid and blood (Daneman et al., 2010). This is consistent with evidence that even early simple endothelial cell sinusoid forming arrays form a 'tight enough' layer to restrict passage of polar molecules as small as sucrose (Saunders et al., 2014). Nevertheless, there is uncertainty in predicting the handling of particular substances where their transport has not been specifically studied.

BBB enzymes such as γ -glutamyl transferase, alkaline phosphatase and aromatic decarboxylase are enriched in cerebral microvessels compared with similar sized vessels elsewhere in the body. They metabolise neuroactive compounds in blood and may be differentially expressed in apical and basal domains (Betz and Goldstein, 1978; Betz et al., 1980).

Altered permeability of the barrier

The BBB usually constitutes an effective barrier to infection and within it is regarded as an immunologically privileged site (Muldoon et al., 2013). Many pathological effects, infectious agents and physiological factors may disrupt BBB function leading to neurotoxicity, encephalitis, meningitis and brain oedema. Some of these have increased incidence in the newborn (Lee et al., 1997; Kimelberg, 2004). Disruption of paracellular pathways (Chen et al., 2003) may be more serious than, for example, pneumolysin effects on transcellular pathways (Nizet et al., 1997). Toxins, viral infections, eukaryotic parasites and fungi penetrate the BBB, rendering it permeable to other agents (Stamatovic et al., 2008). Inflammatory mediators, matrix metalloproteinases, growth factors prostaglandins reactive oxygen and nitrogen affect the function of the BBB (Stamatovic et al., 2008).

It is not totally understood how all the 'opening mechanisms' operate but opening by any means will affect local toxicokinetics and increase CNS exposure to neurotoxins. Some appear to affect only the paracellular route possibly through interleukin 1- β and CXCL8, whereas compounds such as TNF- α increase only transcellular permeability (Butt et al., 1990). Sometimes opening is transient such as when histamine causes intracellular Ca^{2+} accumulation. Thrombin is one rather potent cause of cytoskeletal reorganisation that results in more prolonged opening of the BBB (van Hinsbergh and Amerongen, 2002). This pathway incidentally indicates a theoretical biologically plausible mechanism of damage to the BBB from birth trauma. Highly effective inducers of BBB hyperpermeability other than thrombin (Lee et al., 1997) are amyloid- β peptide (Mackic et al., 1998) and intracellular Ca^{2+} (Brown et al., 2004). Leukocytes in blood may cause BBB permeability and use diapedesis to enter the brain themselves (Alvarez and Teale, 2006). Exogenous pharmacological agents such as nicotine are known to affect the BBB and neonicotinoid pesticides may through their action on intracellular Ca^{2+} also disrupt BBB function. *In vitro* models of the BBB are in development, although no model validated for risk assessment is currently available (Dehouck et al., 1990; Garberg et al., 2005; Culot et al., 2008; Paolinelli et al., 2013).

5.3.2. The developing brain

The development of the human brain starts on day 19 in the human embryo and comprises a number of key stages that progress through the neonatal and infant period well into adolescence before the brain is fully mature (European Medicines Agency, 2009). These steps consist of neuronal proliferation, migration, organisation and myelination. In a first phase, neuronal proliferation and the generation of radial glia occur between gestation weeks 8 and 16, followed by a second phase between 5 months and 1 year of life, consisting of glial multiplication (while neurogenesis and neuroproliferation are continuing). Post-natal brain growth in humans is prolonged and proceeds at fetal growth rates for nearly a year or even longer (Neubauer and Hublin, 2012). As previously reviewed by (EFSA PRR Panel, 2013; ten Donkelaar and Lammens, 2009), the development of the cerebellum is protracted. It commences early in the embryonic period. The development occurs in two waves in humans, the adult number of narrow, leaflike gyri of the cerebellar cortex (referred to as folia or cerebellar laminae) is achieved by around 2 months post-natally and the extragranular layer finally

disappears around the seventh month post-natally. Sensitivity of the cerebellum to neurotoxins in the under 16 week period when it is developing is therefore potentially greater than in older infants and adults.

Neurotransmitters, such as glutamate, γ -aminobutyric acid, dopamine, serotonin, acetylcholine, opiates and adenosine, play a crucial role in the control and modulation of pre- and post-natal brain maturation. For instance, neurons with γ -aminobutyric acid are involved in migrational events following radial glial guides whereas *N*-methyl-D-aspartate receptors transduce excitatory signals in the developing brain, influence synaptic construction, and promote proliferation and migration of neuronal progenitors. The latter events play a crucial role in the development of brain plasticity, which results from a subtle balance between neuronal proliferation and physiological apoptosis.

The vulnerability of the immature brain derives from incomplete myelination and receptor-maturation as well as a lack of endogenous protectors especially in the preterm neonate. This vulnerability can be exacerbated by pre- and post-natal events, including maternal diseases and infections that affect brain maturation.

Increased intracerebral bilirubin concentrations may lead to bilirubin encephalopathy and severe brain damage (kernicterus). The pathogenesis of bilirubin encephalopathy is multifactorial and involves an interaction between unconjugated and free bilirubin levels, albumin binding capacity, acid-base status and neuronal susceptibility to injury (Watchko, 2016).

5.3.3. Summary

The developing BBB is similar to the adult BBB, in that it possesses functional tight junctions and influx and efflux transporters. Hence, contrary to previous assumptions, the healthy BBB in the first 16 weeks, although immature, is considered functional and not leaky. However, the expression/activity of transporters and metabolising enzymes appears to vary from adult patterns to meet the needs of the developing brain and apparently give rise to the measured differences in uptake (Ek et al., 2012) and metabolism rates (e.g. CYP2D6) across the neonatal and infant BBB (Mann et al., 2016). The movement of molecules across the BBBs depends on their physicochemical properties, the local physiological milieu (e.g. pH) and the need for transporters (i.e. influx and efflux transporters) and their ontogeny, activity and specific location. The differences may result in greater vulnerability to chemicals than in the adult (Johansson et al., 2008; Ek et al., 2012; Neal-Kluever et al., 2014).

Uncertainty in predicting specific exposure of the CNS to chemicals derives from knowledge gaps concerning the degree to which alternative pathway barriers such as the blood choroid plexus barrier are functional, precise differentiation state of the BBB.

5.4. The developing immune system

The immune system is a defence system designed to react to exposure to any foreign and 'non self' component. To this aim, a large number of cells and humoral mediators interplay and contribute to an adequate immune response, both by non-specific (innate)- and antigen-specific acquired (or adaptive) immune responses (Delves et al., 2017). In the course of these responses, efficient mediators are released and potent mechanisms are triggered that need to be adequately regulated to avoid any undesirable injury to the responding organism itself, including auto immunity and/or allergy (Delves et al., 2017). Finally, it must be considered that the immune system includes both systemic and local (e.g. lungs, skin, gastrointestinal tract) mechanisms, which may react independently but remain interacting. In this regard, in the neonate the development of the gastrointestinal immune system may be especially vulnerable and deserves a specific attention (Brugman et al., 2015).

At birth, the immune system is far from being completely functional and requires some time before being fully efficient. Newborns mostly rely on their innate (non-specific) immune system as their adaptive (specific) immunity and their mainly Th2 skewed adaptive (specific) immunity fully develop only later in life, as result of a delayed onset of the Th1 immunity and neonatal antibody responses. The imbalance between Th1 and Th2 responsiveness in the newborn is assumed to contribute to the susceptibility of infants to infection and allergic reactions (Dietert and Piepenbrink, 2006; Siegrist and Aspinall, 2009; Zaghoulani et al., 2009; Krishnamoorthy et al., 2012). The development and maturation of the immune system, are initiated *in utero* during the fetal period, and expand in time through the first years of childhood (Pettengill et al., 2014). Accordingly, adequate building up of this system is especially prone to any factor disrupting the numerous mechanisms involved in these processes during the fetal life, in the neonate and the young infant (Gasparoni et al., 2003; Kotiranta-Ainamo et al., 2004; Hartel et al., 2005; Dietert and Piepenbrink, 2006; Velilla et al., 2006; Adkins, 2007; Morein

et al., 2007; Greer et al., 2008; Saito et al., 2008; Belderbos et al., 2009; Dietert, 2009b; Walker et al., 2011; Renz et al., 2012; Sharma et al., 2012; Ygberg and Nilsson, 2012; Neal-Kluever et al., 2014; Valiathan et al., 2016).

In addition, it should be considered that the immune system shares mediators, and therefore interferes with, for example, the endocrine and the nervous systems (Madhusudan et al., 2013; Kabouridis and Pachnis, 2015); consequently, any alteration of one of these mediators may influence the development of any of the systems that share these mediators.

Environmental influences

From a risk assessment standpoint, the issue at hand is whether exposure to immunotoxicants during development and maturation of the immune system produces more severe or persistent health aspects than similar exposure after the immune system is fully mature. It has been reported that adult exposure assessment does not always predict the risk of perinatal immunotoxicity (Dietert, 2009a, 2011; DeWitt et al., 2012; Kollmann et al., 2012; Krishnamoorthy et al., 2012).

Although not established absolutely, it is generally recognised that the immature immune system is more or at least differently (qualitatively) susceptible to immunotoxicants than the fully mature system (Hrube et al., 2005; DeWitt et al., 2012; Kollmann et al., 2012; Krishnamoorthy et al., 2012). Several studies using animal models have shown that the developing immune system is altered by (immuno) toxicants, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), tributyltin or Pb, at doses that were significantly lower than those required to produce effects in the adult. In addition, different effects can be observed depending on the age at which the organism is exposed. For example, tributyltin affects a broader spectrum of immune function upon perinatal than adult exposure, and perinatal TCDD exposure mainly affects cell-mediated immunity (decreased, with males more sensitive than females), whereas adult exposure especially affects humoral immunity (enhanced) (Luebke et al., 2006). Furthermore, sequels of exposure to immune toxicants during development may be particularly persistent in contrast to effects observed following adult exposure, which generally occur at higher doses (Holladay and Smialowicz, 2000).

Maternal influences

Being fed with breast milk in the first weeks of its life is for the newborn a significant opportunity for an efficient and harmonious development and function of its immune system (Marques et al., 2013; Yong et al., 2013). For example, breastfeeding provides the newborn with immunoglobulins and other components or modulators (e.g. fatty acids, oligosaccharides) of the immune system. However, composition of the breast milk can be altered by contamination with substances that may be present in the foods eaten by the mother and that are able to cross from the blood to the milk, and this may result in disturbance of the beneficial properties of breast feeding.

In addition to direct beneficial effects on the immune system itself, breast milk favours an adequate colonisation of the gastrointestinal tract with a microflora that helps in the maturation of the immune system (Parigi et al., 2015). The human gut microbiota, the mixed-species community of microbes that reside in the gastrointestinal tract, plays a critical role in physiological and immunological maturation and homeostasis (Donovan et al., 2012). Epidemiological studies suggest that the common feature of many allergy risk-related exposures is their influence on the founding and early development of a child's gut. Perturbations to gut bacterial community composition during the neonatal period have been associated with a variety of paediatric disorders, highlighting the relationship between early life gut microbiota development and childhood health status. Microbial exposures could alter the risk of subsequent allergic disease and asthma. The human microbiome plays a role in immune regulation, which may impact against the development of allergic diseases (Bengmark, 2013; Alegre et al., 2014; Kabat et al., 2014; Salzman, 2014). In this context, it should be noted that the uterus may also play an indirect role in the immune development through fetal microbial colonisation (Brugman et al., 2015; Romano-Keeler and Weitkamp, 2015).

Allergic reactions

Data on the consequences of exposure to an allergen in infancy are available mainly from studies investigating food allergy prevention involving older infants from the age of 4 months onwards.

The concepts of an 'optimal window' for oral introduction of allergens during which the chance for the development of immune tolerance is greatest, have recently been discussed (Gray, 2015). They were based on studies focussing on the timing of introduction of allergenic food components, which led to a 'paradigm shift' of strategies, away from the earlier concept of 'strict, total allergen avoidance',

towards a concept of 'tolerance induction' by early oral introduction of allergenic foods (de Silva et al., 2014; Tey et al., 2014; Gray, 2015; Perkin et al., 2016). These studies, even if they were not without methodological flaws, suggested that early introduction of allergens (e.g. from egg, wheat or milk), between 4 and 6 months or below 7 months, respectively, may protect against allergies (Poole et al., 2006; Katz et al., 2010; Koplin et al., 2010; Nwaru et al., 2010). Similarly, a randomised control trial on optimal timing of peanut introduction in the infant at risk of peanut allergy (Du Toit et al., 2015) showed that early introduction of peanut significantly decreased the development of peanut allergy amongst children tested negative on a peanut skin prick test and those at high risk for this allergy and modulated the immune response to peanuts. Other studies showed that feeding of hydrolysed or partially hydrolysed cow's milk-formulae led to reduced allergic manifestations and food sensitisations compared to standard cow's milk-based formulae (Muraro et al., 2014; von Berg et al., 2016).

As is reflected by current recommendations supported by the European Academy of Allergy and Clinical Immunology (Muraro et al., 2014), existing information reports that infants at high risk may benefit from reduced exposure to substances being potential allergens in the first 4 months of their lives (Halcken, 2004; Poole et al., 2006; Katz et al., 2010; Koplin et al., 2010; Nwaru et al., 2010; Du Toit et al., 2015).

5.4.1. Summary

The developing immune system in fetal and early post-natal life is particularly sensitive and exposure to immunotoxicants may result in persistent effects on the immune system that last or appear only long after exposure and in general, may also occur at lower doses than during adult exposure. In addition, immune effects seen upon perinatal or adult exposure may be different. A number of compounds or types of exposure may produce different ranges or severities of outcomes depending on the time of exposure during the immune system development. Different and unpredictable arrays of alterations (quantitative and qualitative) may be expected when the exposure occurs *in utero* or in the early neonate versus adult exposure. They may be associated with chronic immunological conditions such as autoimmunity, immune deficiency, inflammation and allergic reactions. Altogether, neonates have specific features in the development and maturation of their immune system, which make their response to an immunogenic/allergenic compound different from that of an adult and therefore, deserve specific studies.

5.5. Development of the reproductive system

Adequate measurements of reproductive function and development include endpoints early in life such as development of sex organs and attainment of pubertal milestones. The reproductive system is heavily influenced by the endocrine system, which facilitates the release of hormones that influence growth and development of many organ systems (Neal-Kluever et al., 2014).

Reproductive organs develop *in utero* and are immature at birth (Lemasters et al., 2000). Some of the critical steps in the formation of the reproductive system take place during embryogenesis. Sexual maturation and related events do not occur until puberty.

5.5.1. Female reproductive organ development

At birth, female infants possess their entire complement of oocytes and further oogenesis does not occur (Neal-Kluever et al., 2014). There are many milestones to accomplish for female reproduction such as maturation of reproductive tissues, steroid hormone production, development of external genitalia, development of sexual behaviour and cyclic hormonal signalling events (Beckman and Feuston, 2003). The period of birth through 6 months is relatively quiescent in terms of female reproductive development, although chemical exposures during this period might have latent effects not detectable until reproductive maturation. For example, girls perinatally (*in utero* and via breastfeeding) exposed to high levels of polybrominated biphenyl, as a result of an accidental contamination with a fire retardant in Michigan in 1973, had an earlier age at menarche compared to breastfed girls exposed to lower levels *in utero* or girls who were not breastfed (Blanck et al., 2000).

5.5.2. Male reproductive organ development

The first 6 months after birth are considered a sensitive developmental window for the testes (Lemasters et al., 2000). The early post-natal testis exhibits a high proportion of immature Sertoli cells (Mendis-Handagama and Ariyaratne, 2001) that proliferate from birth until 6 months of age when the

adult number of Sertoli cells is achieved (Lemasters et al., 2000). The number of Sertoli cells that develop during this period directly influence the number of spermatogonial cells that can be supported (Cortes et al., 1987), and hence this heavily affects sperm count and fertility later in life (Lemasters et al., 2000). Furthermore, the infant testes have a higher susceptibility for toxic injury compared to the adult because the blood–testis barrier does not fully develop until puberty (Camatini et al., 1981). There is one major androgen surge during human male development, namely from 4 to 6 weeks of gestation, followed by a secondary surge shortly after birth. Leydig cells undergo a rapid period of growth around the third or fourth month after birth (Codesal et al., 1990; Mendis-Handagama and Ariyaratne, 2001), which is related to a transient increase in activity of the hypothalamic–pituitary–gonadal (HPG) axis (Mann and Fraser, 1996; Prince, 2001).

5.5.3. Summary

The development of the female reproductive system in infants below 16 weeks of age is relatively quiescent. For the development of the male reproductive organ the testis; however, this is a sensitive period. The early postnatal testis exhibits a high proportion of immature Sertoli cells (Mendis-Handagama and Ariyaratne, 2001), which proliferate from birth until 6 months of age when the adult number of Sertoli cells is achieved (Lemasters et al., 2000). Effects on the functional development of reproductive organs generally become apparent later in life.

5.6. Development of the endocrine system

5.6.1. The hypothalamic–pituitary–gonadal (HPG) axis

In humans, the HPG axis develops *in utero* by the end of the first trimester (Mann and Fraser, 1996; Grumbach, 2002). Thus, the components of the HPG axis are present at birth. In the first 6 months after birth, there are transient increases in follicle-stimulating hormone (females) and luteinising hormone (males), followed by a period of diminished activity that lasts until puberty (Grumbach, 2002). There are some indications from observational studies that the HPG axis may be disrupted by chemicals that result in altered puberty (Blanck et al., 2000; Ouyang et al., 2005; Den Hond and Schoeters, 2006; Louis et al., 2008; Korrick et al., 2011; Maranghi and Mantovani, 2012; Deodati et al., 2016). Although changes in the onset of puberty have been associated with exposure to several chemicals, the underlying mechanism remains unknown.

Minipuberty is the name given to the activation of the HPG axis. It is described in detail and summarised here because it coincides with the period of concern for this risk assessment (Kuiri-Hänninen et al., 2014). From midgestation, the HPG axis is active. Near to term, placental hormones cause its activity to be suppressed. Once the umbilical cord is cut at birth, the placental hormones no longer reach the early infant's body, and reactivation of the HPG axis increases circulating gonadotrophin levels. Gonadotropin levels are still high during the first 3 months of life but decrease towards the age of 6 months (exceptionally follicle-stimulating hormone levels in girls remain high until 3–4 years). After this, the HPG axis remains inactive until puberty. The early post-natal availability of gonadotropins mediates gonadal development. Male babies show testosterone levels peaking at 1–3 months of age then declining following luteinising hormone levels, and this is important for proper development of male genitalia. High gonadotropin levels in female babies promote maturation of ovarian follicles and raised oestradiol levels.

Minipuberty is an under-researched area and knowledge of the mechanisms that suppress the HPG axis until puberty is limited. Such data gaps contribute to uncertainty in risk assessment. However, it is clear that the first post-natal months of life provide targets for endocrine active substances (EFSA Scientific Committee, 2013) that may have different endocrine mediated outcomes from those later in development and in adulthood.

5.6.2. The fetal and infant adrenal glands

Development of the adrenal glands starts by day 25 of gestation. The fetal adrenal glands function in cooperation with the placenta. By the fourth month of gestation, they are four times the size of the kidney but, by birth, following a rapid decline of the fetal adrenal cortex, they are only one-third the size of the kidney. Through apoptosis, the fetal adrenal cortex disappears almost completely by the end of the first year. At 1 year, a healthy human adrenal gland weighs approximately 1 g, but then increases to a final weight of 4–5 g when it is fully developed at 4–5 years (Ishimoto and Jaffe, 2011).

These rapid changes from the fetal to the final form of the adrenal cortex occur in the time window of interest for this risk assessment. Premature babies, stillborn and some neonates that have experienced intrauterine stress have microscopic cystic changes in adrenal glands an observation that adds uncertainty to the risk assessment of substances found in food intended for preterm and term infants.

5.6.3. Adrenarchy and the hypothalamic–pituitary–adrenal (HPA) axis

As indicated above, effects at any early developmental stage may be reflected in deficits at later time points. At adrenarchy, the preparation for puberty, there is an increase in adrenal androgen production by the zona fasciculata of the adrenal gland. This is biochemically detectable as a rise in dehydroepiandrosterone sulfate (DHEA-S) commencing at approximately 6 years of age in both sexes. It is a response to adrenocorticotrophic hormone that causes a greater rise of 17-hydroxypregnenolone and DHEA than the simultaneous rise of cortisol. The unique pattern of steroidogenic enzymes that accompanies these changes includes expression of sulfotransferase 2A1 (a catalyst of the formation of DHEA-S), which is the major adrenarchal steroid.

The onset of puberty varies across populations and sexes and is influenced by nutritional status. Breast budding is normal in Afro-American and Mexican girls as early as 7 years (Rosenfield et al., 2009) but good data for the onset of male puberty is still unavailable (Bordini and Rosenfield, 2011). Premature or precocious puberty is defined as onset 2 to 2.5 standard deviations earlier than population norms. Precocious puberty is attributable to androgen and/or oestrogen effects. These may be centrally mediated through the HPG axis, or in 'peripheral' precocious puberty from an autonomous peripheral origin such as the adrenal glands, gonads, germline or neural crest derived tumours. It may also have an exogenous basis. In boys with precocious puberty, where both testes are enlarged this may be in response to luteinising hormone. This condition is HPG related or 'central' precocious puberty. It could also be the result of a human chorionic gonadotropin-secreting tumour. Where both testes are pre-pubertal in size, the most likely source of the androgens is adrenal. The exogenous causes include topical exposure to lavender and tea-tree oil. Here, the effects are assumed to depend on the anti-androgenic effects of these compounds which caused gynaecomastia in pre-pubertal boys (Henley et al., 2007). Similarly, oestradiol in an Evamist spray, a menopausal symptom relief prescription treatment, may cause precocious puberty in girls, gynaecomastia in boys and may affect mammary glands in companion animals when inadvertent skin contact is made.¹¹

5.6.4. The fetal and infant thyroid and parathyroid glands

The serum half-life of T4 is approximately 7–10 days in adults (Sabatino and Chopra, 1999), but is approximately 3 days in neonates (Lewander et al., 1989; van den Hove et al., 1999). Thus, the T4 secretion from the thyroid gland has to be considerably higher in early life both to maintain steady-state levels and to satisfy the requirements of the growing/developing newborn.

Moreover, the adult thyroid gland stores a large quantity of thyroid hormone in the form of thyroglobulin, which is sufficient to maintain normal levels of circulating hormone for several months (Greer et al., 2002). By contrast, the neonatal gland stores very little T4; the amount stored has been estimated at less than that required for a single day (van den Hove et al., 1999; Savin et al., 2003). These differences indicate that the functional reserve available to adults is virtually absent in neonates; consequently, any reduction in thyroid hormone synthesis in the neonate might result in a reduction in circulating levels leading to hormone insufficiency.

The thyroid has a key role in many developmental processes. For example, in skeletogenesis, thyroid hormone receptors (TR α 1 and TR β 1) are functional in growth plate hyaline cartilage cells, osteoblasts and bone marrow stromal cells. T3 binds to TR α 1 and regulates endochondral ossification through signalling pathways such as the Indian hedgehog/parathyroid hormone related peptide feedback loop and the growth hormone insulin-like growth factor 1 and fibroblast growth factor receptor pathways (Braverman and Cooper, 2012). These processes are initiated as different ossification centres become active and continue until growth plate closure is complete. The process of ossification is extended: as examples, six ossification centres that appear during gestation and the neonatal period can be considered. They develop in the order calcaneum, talus, distal femur, cuboid bone and proximal humerus. The first of these appear at week 21 and all six are usually present by weeks 41–42 (Pryse-Davies et al., 1974). The time of ossification is a major difference between the early infant and premature baby and an adult and allows for differences in response to toxicants.

¹¹ <http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm220185.htm>

Interindividual variation in the timing of the onset of ossification does add uncertainty to defining windows of temporal sensitivity to development of different parts of the skeleton as there is a wide range of times of their first appearance. Retardation and advancement are both correlated with other factors. Retardation is associated with male sex, small-for dates babies, some congenital malformations, multiple births, increasing maternal age and increasing parity. The thyroid plays also a key role in neurological development, metabolism regulation, bone remodelling and other essential functions (Boas et al., 2012). Disruptions in thyroid signalling can have widespread effects on infant development (Zimmermann, 2011).

The structure and development of the parathyroid glands, of which four are usually found in humans and most mammals, are well described in a recent publication (Kalinin et al., 2013). The glands develop prenatally and are essential for skeletal growth during early post-natal development.

Parathyroid hormone (parathormone) is a peptide that controls the blood levels of calcium and phosphate and hence also bone physiology. Its effects are antagonistic to those of thyroid derived calcitonin, which downregulates osteoclasts and is most active during lactation and pregnancy preventing excessive bone demineralisation. Parathyroid hormone increases gastrointestinal calcium absorption by activating vitamin D, and promotes calcium conservation (reabsorption) by the kidney. It is also a major regulator of serum phosphate concentrations by the kidney. It is an inhibitor of proximal tubular reabsorption of phosphorus. Through activation of vitamin D the absorption of phosphate is increased (David and Anast, 1974).

Parathyroid secretion is normally low in the early new born period. It increases rapidly 48 h after birth. The net effect of unknown plasma hypocalcaemic factor(s) on the one hand and parathyroid activity on the other may account for differences in plasma Ca^{2+} levels observed between normal and hypocalcaemic infants. Low levels of Mg^{2+} are frequently present in hypocalcaemic infants. Hypomagnesemia may reflect parathyroid insufficiency or the converse (David and Anast, 1974) adding uncertainty to predicting the effects of substances at this early life stage.

Taken together, the thyroid and parathyroid glands are independent organs, each having specific functions. This, however, does not exclude the occurrence of a direct or indirect interaction in the functions of the two systems. There is reason to believe that an insufficiency of the parathyroid gland downregulates to some extent the function of the thyroid gland (Tanberg, 1916; Castro et al., 1975).

5.6.5. Summary

Infants below 16 weeks of age have specific endocrine profiles that are different from those in adults. This suggests that such differences may require particular attention for the risk assessment of substances present in food intended for infants below 16 weeks of age. Effects at any early developmental stage may be reflected in deficits at later time points, indicating that the window of exposure, the window of maximum sensitivity and the window of effect require a lifespan approach to generate a fully protective risk assessment. This is because the disruption of the programming role of hormones during post-natal development can potentially cause adverse effects that do not become evident until later in life. Although our scientific knowledge in this area is developing rapidly, concerted research and development efforts are still needed to fill existing knowledge gaps (Solecki et al., 2017).

6. Considerations of testing strategy for substances present in food intended for infants below 16 weeks of age

Testing strategies for substances present in food intended for infants need to take into consideration the differences in development stages of the relevant organ systems and the related critical windows of maximum sensitivity between humans and laboratory animals used in safety testing. Information on comparative differences in the post-natal development of organ systems and physiological processes between humans and commonly used experimental animal models can be found in the literature (WHO, 2006; Office for Risk Assessment, 2008; Beck et al., 2016; Kuper et al., 2016).

6.1. Information provided by standard testing of substances present in food

The safety evaluation of substances that may be present (intentionally or not) in the foods consumed by infants below the age of 16 weeks should follow the general principles set for the safety evaluation processes used for substances present in food intended for the general population and

should make use of information already obtained in standard toxicological studies following international guidelines for toxicity testing. The testing programmes for the safety evaluation of substances present in food used for the general population will depend on the applicable guidances¹² and legislations.¹³ Typically, these studies will provide information on ADME, subchronic and chronic toxicity, genotoxicity, carcinogenicity and reproductive toxicity.

6.2. Additional information needed when testing substances present in food intended for infants below 16 weeks of age

In view of the potential greater sensitivity during the first few weeks of life, the EFSA SC considers that additional studies may be necessary for the risk assessment of substances intended to be consumed by this population. These are detailed below.

When standard toxicological studies following international guidelines for toxicity testing do not show adverse effects and the ADME studies show that a substance is not absorbed in relevant amounts, only an additional repeated dose study with direct oral administration to neonatal animals (e.g. in piglet models) is needed. This latter study should include analysis of possible local effects on the gastrointestinal tract and on a possible reduction in the bioavailability of nutrients (minerals and vitamins) that are normally contained in food for infants.

When standard toxicological studies do not show adverse effects and the ADME studies show that a substance or its metabolite(s) is (are) absorbed, the approach as described below should be followed.

An Extended One-Generation Reproductive Toxicity Study (EOGRTS) (OECD TG 443) is warranted. Following the EOGRTS guidelines, rats are the species of choice and the pups are (possibly) exposed *in utero*, during lactation and after weaning, until at least post-natal day 42. For the purpose of this guidance, three cohorts of F1-animals (as described in the OECD TG 443) should be included: a cohort to assess the reproductive and developmental endpoints, a cohort to assess the potential impact of a test substance on the developing nervous system, and a cohort to assess the potential impact on the developing immune system. Because exposure through mother's milk in experimental studies is usually substantially lower than the exposure through feed, the resulting doses in the neonatal animals may be insufficient for hazard characterisation. Therefore, for the purpose of this guidance, it is advised that direct dosing of the neonatal animals should be considered as soon as possible after birth.

In case scientific justification is given, it is possible to deviate from the guidance given here.

For example, when a two-generation reproductive toxicity study (OECD TG 416) is available instead of an EOGRTS, the former could be complemented with appropriate post-natal development studies addressing (e.g. neurotoxicity and immunotoxicity). In some cases, additional studies in neonatal animals in a second non-rodent species (e.g. the piglet models), specific *in vitro* assays or mechanistic studies may be needed.

Other information

Human data (epidemiological, clinical studies and case reports, where available) should be included in the evaluation. The general principles underpinning the risk assessment performed according to the guidance or recommendations established by the different EFSA panels and units should be applied.

6.3. Piglet models for toxicity testing of substances present in food intended for infants below 16 weeks of age

Piglet models, including neonatal mini piglets or neonatal farm piglets, are frequently used as an animal model in safety and efficacy research of chemicals and pharmaceuticals as they closely resemble humans in anatomy, physiology and biochemistry and because of the practical features of toxicity testing in piglet models (e.g. Bode et al., 2010). In particular, the gastrointestinal tract, the

¹² See e.g. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Guidance for submission for food additive evaluations. EFSA Journal 2012;10(7):2760. [60 pp.] <https://doi.org/10.2903/j.efsa.2012.2760>; EC (European Commission), 2001. Guidance of the Scientific Committee on Food (SCF) for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation. SCF/CS/PLEN/GEN/100 Final. 19 December 2001. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. EFSA Journal 2016;14(11):4594, 24 pp. <https://doi.org/10.2903/j.efsa.2016.4594>

¹³ See e.g. COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

cardiovascular system, the skin, the urogenital system, metabolic aspects and the immune system of pigs are considered generally more like humans than those of other non-rodent species (Helm et al., 2007; Guilloteau et al., 2010; Barrow, 2012; Heinritz et al., 2013; Odle et al., 2014). As in pigs no transplacental transfer of antibodies exists, (pre)term piglets need to stay for the first 24–48 h with their mother for obtaining passive immunological protection by antibodies via mother's colostral milk (Farmer and Quesnel, 2009; Penninks et al., 2012), or by intra-arterial injection within the first 24 h of mother's plasma to obtain immunological protection (Hansen et al., 2016). In addition, direct oral administration of substances, including bottle-feeding, can be performed in this model. Therefore, the (pre)term piglets can be considered as a relevant animal model for the safety evaluation of food additives, pesticide residues and contaminants present in food (i.e. via pig-adapted infant formula) on general toxicity parameters (i.e. growth, food/water consumption, haematology and clinical pathology, toxicokinetics, organ weights and histopathology evaluations, etc.) and post-natal maturation and development of various organ systems, such as the gastrointestinal tract (Van Ginneken, 2012; Sangild et al., 2013), the metabolic and renal capacities (McAnulty, 2012; Preusse and Tingleff Skaanild, 2012), the immune- and the reproductive systems (Haley, 2012; McAnulty et al., 2012), and the nervous system (Lind et al., 2007).

As pre-term piglets delivered at 90% gestation suffer from many of the same disorders and physiological problems as moderately immature human infants, they may comprise a relevant model for studying the safety evaluation of early dietary interventions with food additives, pesticide residues and contaminants present in the food of the preterm piglets and on e.g. microbiota-related complications (Barrow, 2012).

Vaginally delivered neonatal piglets are described as a model for investigating the influences of substances added to infant formulae on the development of the infant microbiota among other parameters of intestinal functions (Alizadeh et al., 2015; Hoefflinger et al., 2015). More realistic testing conditions are generated using interspecies transplantation of gut microbiota from the human adult and infant gastrointestinal tract to that of piglets (Pang et al., 2007; Zhang et al., 2013; Wang and Donovan, 2015).

6.4. Testing of the developing immune system

Despite the need for developmental immunotoxicity testing, there are no validated or widely accepted methods for evaluating effects on the developing immune system, but there is general consensus that when possible, methods to assess developmental immunotoxicity testing (DIT) should be included in existing developmental and reproductive toxicology (DART) protocols (Holsapple et al., 2005; Ladics et al., 2005; Cooper et al., 2006; Dietert and Holsapple, 2007; Burns-Naas et al., 2008; DeWitt et al., 2012).

When testing the developing immune system, it is important to account for the fact that there are different endpoints in the development of the immune system and in their maturation rates (US FDA, 2006). Integration of parameters reflecting the immune system in reproductive or developmental guidelines is minimal to date and it is limited to the EOGRTS guideline (OECD TG 443, 2011). This guideline is designed to provide an evaluation of the pre- and post-natal effects of chemicals on development, as well as a thorough evaluation of systemic toxicity in pregnant and lactating females, and also young and adult offspring, and includes additional endpoints for DIT as well as developmental neurotoxicity. The DIT default parameters include the standard immune pathology parameters and as this might not always be sensitive enough (Bunn and Dietert, 2001), an immune functional assay is also included in this EOGRTS guideline. The functional assay included is a T-cell-dependent antibody response with sheep red blood cells as a particulate antigen or keyhole limpet haemocyanin as a protein antigen. Although not included in the EOGRTS guideline, additional functional assays might be considered (Bunn and Dietert, 2001).

6.5. Testing of the developing nervous system

When testing the developmental nervous system to assess the safety of substances added to foods for infants, the developmental neurotoxicity study under OECD TG 426 or as part of EOGRTS can be used. Direct oral dosing of the pups should be considered. It was noted that the current data requirements for developmental neurotoxicity testing may not always be sufficient to identify all potentially hazardous compounds and that additional information may be provided by appropriate *in vitro* testing batteries (EFSA PRR Panel, 2013; Fritsche et al., 2017).

6.6. *In vitro* assays

There is a scarcity of validated lower tier *in vitro* screening tests that are applicable to the first 16 weeks of post-natal human development. There are opportunities to develop such tests that would be relevant for example to measurement of uptake across the early gut and the brain barriers at early life stages. Likewise, there is a need for appropriately triggered developmental neurotoxicity tests. A recommendation follows that the existing test systems should be further developed to provide *in vitro* tools that can efficiently pre-screen substances as part of a comprehensive risk assessment approach for substances in food for infants below 16 weeks of age.

7. Approach for the risk assessment of substances present in food intended for infants below 16 weeks of age

In general, it is assumed that effects observed in adults are also expected to occur in infants, which is supported by the available evidence. The reverse may not always be the case (e.g. the developing brain may be specifically affected or disruption of the programming role of hormones during post-natal development may cause adverse effects that do not become evident until later in life). Most differences between infants and adults are of a quantitative nature, i.e. the effects may occur at lower or higher doses than in adults. Appropriate developmental studies may reveal such effects for specific targets in that age group.

It is often assumed that the young infant is generally more sensitive than the adult. This may not always be true, and the available evidence should be considered on a case-by-case basis.

Because considerable development is taking place in early infancy (particularly in preterm infants) and because milk/formula consumption per kg body weight is high, and hence exposure, is greatest in the first month of life, the period of potentially greatest sensitivity is the first few weeks. Moreover, this population is heterogeneous because the post-natal age may not be representative of the actual development of the newborn, specifically in pre-term born infants.

A decision tree to decide on the approach for the risk assessment of substances present in food intended for infants below 16 weeks of age is presented in Figure 7. It enables a case-by-case risk assessment taking into consideration existing data including whether relevant amounts of the substance are absorbed and all relevant data.

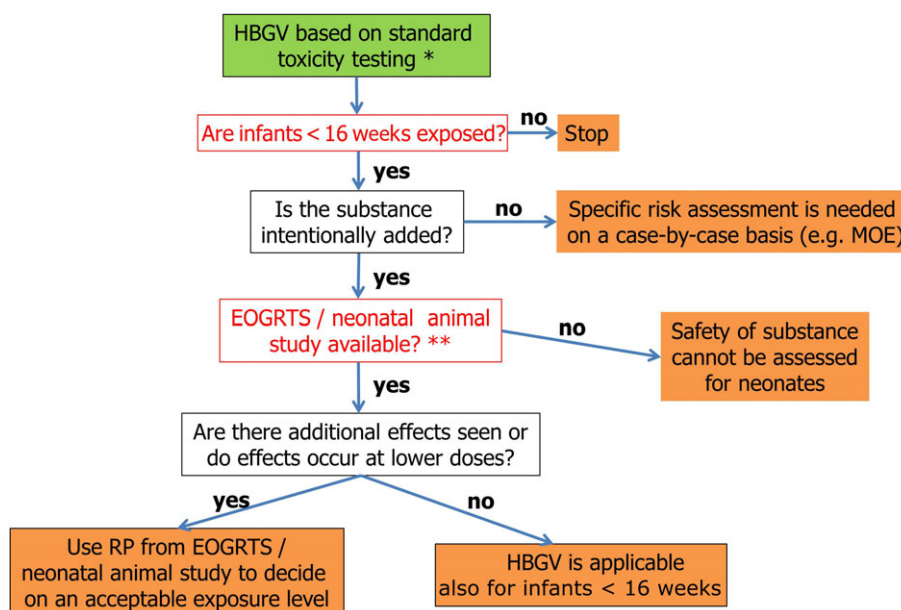


Figure 7: Decision tree approach for the risk assessment of substances present in food intended for infants below 16 weeks of age

RP: reference point

*: This decision tree assumes that a standard chemical risk assessment has already been performed on the substance of interest. Note that standard toxicity testing varies in the different areas within the remit of EFSA.

** : Extended one-generation reproductive toxicity study (EOGRTS) if the substance is systemically available, neonatal animal study if the substance is not absorbed from the gastrointestinal tract and is not systemically available. In case scientific justification is given, it is possible to deviate from the guidance given here (see Section 6).

7.1. Considerations for the risk assessment of substances not intentionally added to food intended for infants below 16 weeks of age

The presence of contaminants in food for infants should be avoided as much as possible.

Ideally, toxicity data from neonatal animal studies should be part of the body of information needed for the risk assessment for substances not intentionally added to food intended for infants below 16 weeks of age. However, such data might not always be available. The following section describes how knowledge on toxicokinetic and toxicodynamic considerations can be applied to support the risk assessment of unavoidable contaminants when data on their toxicity in infants are not available but some data from standard toxicological studies in animals are present.

Our understanding of the maturation of the gastrointestinal tract and the brain barriers has changed; these are now no longer considered as immature or leaky but as functional. Hence, the gut and the brain barriers can be considered to protect the young infant at a level that may be comparable to that seen in older infants and adults. However, it remains clear that developing organs such as the brain and the reproductive and immune systems may be more sensitive to harm in the infant population below 16 weeks of age as compared to the rest of the population.

The impact of the differences in toxicokinetic and toxicodynamic properties of substances in food between infants under the age of 16 weeks and older infants or adults and potential strategies on how to consider them in a risk assessment context are presented below.

Considerable knowledge of the ontogeny of the excretory pathways, specifically the metabolising enzymes, permits now to predict the toxicokinetic behaviour of substances if their metabolism in the adult is known. Even if their metabolic fate is not known in detail, the overall potential impact of the toxicokinetic variability in infants can be addressed. To account for the variability in toxicokinetics in the human population a kinetic subfactor of 3.2 is applied as a default value for the derivation of a HBGV. This factor is in general appropriate. However, the differences between the adult population and

the infants, in particular the very young ones, are in many cases greater than the factor of 3.2 and a larger factor may be necessary (see Section 5 and Appendix C).

Most differences between infants and adults are of a quantitative nature, and therefore, lower or higher doses of a chemical may be needed to cause adverse effects in infants as compared to adults. However, our current knowledge of the toxicodynamic variability in infants does not allow us to predict the magnitude of the difference in sensitivity between young infants and older infants or adults. Furthermore, while adverse effects observed in adults are also expected to occur in infants, the lack of observed adverse effects in adults (under standard test conditions) does not necessarily rule out a potential specific toxicity response in young infants to the same substance. Organs with critical developmental stages during early infancy, such as the developing brain and the immune system, may be affected specifically. Appropriate developmental studies, as described above, may not be available.

Taking into consideration that our current understanding of the toxicodynamic variability in infants is insufficient to make general quantitative and qualitative predictions of adverse effects in infants, the EFSA SC concludes that the uncertainty surrounding the toxicodynamic variability in infants did not allow identifying appropriate additional toxicodynamic default UFs. However, additional considerations on a case-by-case basis are needed to decide whether there is a health concern. In case studies on neonatal animals are available, these are expected to address the variability discussed above.

8. Conclusions

The EFSA SC notes that during the period from birth up to 16 weeks, infants are expected to be exclusively fed on breast milk and/or infant formula. The EFSA SC views this period as the time where HBGVs for the general population do not apply without further considerations.

In its approach to develop this guidance, the EFSA SC has taken into account the following:

- for the exposure assessment, it is taken that in non-breastfed infants, formula is expected to be the only source of nutrition for the first 16 weeks of life;
- the overall toxicological profile of the substance identified through the standard toxicological tests, including critical effects;
- toxicokinetic data, especially:
 - the absorption of the substance from the gastrointestinal tract
 - impaired renal excretion
 - metabolism in the neonatal organism compared with the adult;
- knowledge of organ development (critical windows) in human infants;
- the effects of exposure of neonatal animals at equivalent life stages of human infants;
- the relevance for the human infant of the neonatal animal models used and of the reported effects in toxicological testing;
- whether additional relevant effects are revealed or whether the effects occurred at lower doses in the neonatal animals, by comparison with standard toxicological studies.

The EFSA SC concludes the following:

- 1) High infant formula consumption per body weight is derived from 95th percentile consumption and the first weeks of life is the time of the highest relative consumption on a body weight basis. Therefore, when performing an exposure assessment, the EFSA SC proposes to use the highest consumption figures reported for the period of 14–27 days of life (i.e. 260 mL/kg bw per day). This approach would cover acute toxicity and consider potential periods of high sensitivity for other toxicity endpoints.
- 2) A decision tree approach is proposed that enables a case-by-case risk assessment of substances present in food intended for infants below 16 weeks of age.

9. Recommendations

The EFSA SC recommends that:

- 1) Contemporary data on high food consumption during the first weeks of life after birth are generated.
- 2) Accurate data on exposure to substances present in food during this time period are generated.

- 3) Information from neonatal animal studies would reduce uncertainty when evaluating substances not intentionally added to food (e.g. contaminants).
- 4) Guidance should be developed on how to perform studies in neonatal animals.
- 5) Widely acceptable models should be developed to measure antinutritional effects of substances in food intended for infants below 16 weeks of age.
- 6) There is a need to develop validated *in vitro* tests applicable to the first 16 weeks of post-natal human development that could provide tools to efficiently prescreen substances as part of a comprehensive risk assessment and to improve understanding of the molecular events underlying adverse outcomes.
- 7) Post-marketing studies monitoring potential adverse effects (e.g. direct effects on the gastrointestinal tract) should be made available to reduce uncertainty for substances intentionally added to food.

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Abbreviations

ABC	adenosine triphosphate binding cassette
ADI	acceptable daily intake
ADME	absorption, distribution, metabolism and excretion
ARfD	acute reference dose
ATP	adenosine triphosphate
ATS	apical tubular system
AUC	area under the curve
BB	brain barrier
BBB	blood-brain barrier
bw	body weight
CNS	central nervous system
CONTAM	EFSA Panel on Contaminants in the Food Chain
CSF	cerebrospinal fluid
CYP	cytochrome P 450
DART	developmental and reproductive toxicology
DHEA	dehydroepiandrosterone
DHEA-S	dehydroepiandrosterone sulfate
DIT	developmental immunotoxicity testing
EMA	European Medicines Agency
EOGRS	extended one generation reproductive toxicity study
ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FAO	Food and Agriculture Organization
FCM	food contact materials
FMO	flavin-containing mono-oxygenase
GA	gestational age
GFR	glomerular filtration rate
GNPD	Global New Products Database
HBGV	health-based guidance value
HPA	hypothalamic–pituitary–adrenal
HPG	hypothalamic–pituitary–gonadal
IOM	Institute of Medicine of the US National Academies of Sciences

IPCS	International Programme on Chemical Safety
ISF	infant scaling factor
JECFA	Joint FAO-WHO Expert Committee on Food Additives
K_m	Michaelis–Menten constant
MOE	margin of exposure
MRP	multidrug resistance protein
NOAEL	non-observed-adverse effect
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
PB-PK	physiologically based pharmacokinetic
PK	pharmacokinetic
PoD	point of departure
RP	reference point
SC	Scientific Committee
SCF	Scientific Committee on Food
SLC	solute carrier
SULT	sulfotransferases
TCDD	2,3,7,8-tetrachlorodibenzo-para-dioxin
TDI	tolerable daily intake
TPMT	thiopurine S-methyltransferase
UDP	uridine diphosphate
UF	uncertainty factor
UGT	UDP-glucuronosyltransferase
V_{max}	maximum rate of metabolism
WHO	World Health Organization

Appendix A – Overview of EU Legislation (Food Sector) covering food for infants (and young children)

Glossary

- Infants – Children under the age of 12 months (as defined in Regulation (EU) No 609/2013)
- Young children – Children aged between 1 and 3 years (as defined in Regulation (EU) No 609/2013)

FOOD ADDITIVES

Annex II to Regulation (EC) No 1333/2008 on food additives:

Specific uses of food additives in food for infants and young children (food category 13.1 of Annex II) and specifically from birth onwards and/or for infants below 16 weeks → additives presently authorised in food categories '13.1.1 Infant formulae' and 13.1.5.1 and 13.1.5.2 (as follows)

Infant formulae as defined in Regulation (EU) No 609/2013*

Food intended for use by infants during the first months of life and satisfying by itself the nutritional requirements of such infants until the introduction of appropriate complementary feeding

Follow-on formulae as defined in Regulation (EU) No 609/2013*

Food intended for use by infants when appropriate complementary feeding is introduced and which constitutes the principal liquid element in a progressively diversified diet of such infants

**Commission delegated Regulation (EU) 2016/127 was adopted on 25 September 2015 and will start to apply on 22 February 2020. Until that date, the rules of Directive 2006/141/EC remain applicable*

Processed cereal-based foods and baby foods for infants and young children as defined in Regulation (EU) No 609/2013**

Food intended to fulfil the particular requirements of infants in good health while they are being weaned and by young children in good health as a supplement to their diet and/or for their progressive adaptation to ordinary food

***Until the finalisation of the delegated act, the rules of Directive 2006/125/EC remain applicable*

Other foods for young children

(13.1.5) Dietary **foods for infants and young children for special medical purposes** as defined in Regulation (EU) No 609/2013*** **and special formulae for infants**

****Commission delegated Regulation (EU) 2016/128 was adopted on 25 September 2015 and will start to apply on 22 February 2019. Until that date, the rules of Directive 1999/21/EC remain applicable*

(13.1.5.1) Dietary **foods for infants for special medical purposes** and special formulae for infants

Examples of specific uses of food additives:

From 4 months onwards in special food products with adapted composition, required for metabolic disorders and for general tube-feeding

From 12 months onwards in specialised diets intended for young children who have cow's milk intolerance or inborn errors of metabolism

From birth onwards:

- in products for reduction of gastroesophageal reflux
- in products in liquid formulae containing hydrolysed proteins, peptides or amino acids
- for use in products based on amino acids or peptides for use with patients who have problems with impairment of the gastrointestinal tract, protein malabsorption or inborn errors of metabolism
- in products used in case of gastrointestinal disorders
- in products for the dietary management of metabolic disorders
- in specialised diets, particularly those devoid of proteins

(13.1.5.2) Dietary **foods for babies and young children for special medical purposes** as defined in Directive 1999/21/EC

Examples of specific uses of food additives:

From 4 months onwards in special food products with adapted composition, required for metabolic disorders and for general tube-feeding

From 12 months onwards in specialized diets intended for young children who have cow's milk intolerance or inborn errors of metabolism

From birth onwards:

- in products for reduction of gastro-oesophageal reflux
- in products in liquid formulae containing hydrolyzed proteins, peptides or amino acids
- for use in products based on amino acids or peptides for use with patients who have problems with impairment of the gastrointestinal tract, protein mal-absorption or inborn errors of metabolism
- in products used in case of gastrointestinal disorders
- in products for the dietary management of metabolic disorders
- in specialized diets, particularly those devoid of proteins

PESTICIDE RESIDUES

Regulation (EU) No 609/2013 on food intended for infants and young children, food for special medical purposes, and total diet replacement for weight control

General requirement: Article 11(1)(b) – specific requirements on pesticides for infant formula, follow-on formula, processed cereal-based food, baby food and food for special medical purposes developed to satisfy the nutritional requirements of infants and young children should be updated regularly and include, *inter alia*, provisions to restrict the use of pesticides as much as possible

Directive 2006/141/EC **Infant formulae** shall not contain residues of individual pesticides at levels exceeding 0.01 mg/kg of the product as proposed ready for consumption or as reconstituted according to the instructions of the manufacturer. Lower MRLs apply for specific pesticides listed in Annexes VIII and IX of Directive 2006/141/EC (the same rules are laid down in Commission delegated Regulation (EU) 2016/127 that will start to apply on 22 February 2020. Until that date, the rules of Directive 2006/141/EC remain applicable)

Follow-on formulae (same rules as for Infant formulae)

Directive 2006/125/EC **Processed cereal-based foods** (same rules as for Infant formulae and Follow-on formulae) (until the finalisation of the delegated act, the rules of Directive 2006/125/EC remain applicable)

Baby foods (same rules as for Processed cereal-based foods)

Delegated Regulation (EU) 2016/128: food for special medical purposes intended for infants and young children shall comply with the same rules laid down for formulae and baby foods (from 22 February 2019)

CONTAMINANTS

Regulation (EEC) No 315/93 laying down Union procedures for contaminants in food

Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs

Maximum levels for certain contaminants in foodstuffs:

- nitrate, mycotoxins, metals, polycyclic aromatic hydrocarbons and tropane alkaloids in processed cereal-based foods and baby foods for infants and young children
- mycotoxins, metals, polycyclic aromatic hydrocarbons, melamine and inherent plant toxins in infant formulae and follow-on formulae
- mycotoxins, metals, polycyclic aromatic hydrocarbons in foods for special medical purposes intended specifically for infants
- metals in drinks for infants and young children labelled and sold as such, in rice destined for the production of food for infants and young children
- dioxins and polychlorinated biphenyls in foods for infants and young children

FOOD CONTACT MATERIALS

[Regulation \(EU\) No 1935/2004](#) on materials and articles intended to come into contact with food >> sets out the general principles of safety and inertness for all Food Contact Materials (FCMs)

[Regulation \(EU\) No 10/2011](#) on plastic materials and articles intended to come into contact with food

Maximum levels of specific migration limit for certain substances used in FCM for food for infants and other restrictions:

- epoxidised soybean oil in polyvinyl chloride gaskets used to seal glass jars containing infant formulae and follow-on formulae or processed cereal- based foods and baby foods for infants and young children
- bisphenol A not to be used for the manufacture of polycarbonate infant feeding bottles

Appendix B – Key milestones in human development

Developmental milestone	Gestational age (weeks GA) and post-natal (weeks PN)	Start time (fertilisation age in days: FA)	Completion time (fertilisation age in days: FA)
Fertilisation	2 weeks GA	0	1
Extreme preterm ^(a)	< 28 weeks GA	140	180
Very preterm	< 32 weeks GA	180	210
Moderate preterm	< 37 weeks GA	210	245
Perinatal period	22 weeks GA to 1 week PN	155	280
Normal term birth ^(b)	40 weeks GA to 0 weeks PN	270	1
Neonate/newborn infant	0–4 weeks PN	270	300
Post-term pregnancy	0–4 weeks PN	270	298
Post-natal/post-partum/puerperium	0–6 weeks PN	270	310
Exclusive breast feeding recommended	0–26 weeks PN	270	450
Infant	0–52 weeks PN	270	635

(a): Limit of viability is considered to be 22 weeks GA or birthweight < 500 g.

(b): Less than 5% of births occur at precisely 40 weeks of gestational age. Some 50% are born within 1 week of this; 80% are born within 2 weeks (Kieler et al., 1995). The variation has to be considered as introducing uncertainty into the risk assessment for toxicity at different time windows of development.

Explanatory notes (see also WHO, UNICEF, USAID, AED, UCDAVIS and IFPRI, 2008)

- 1) The timing of birth is variable and several systems have been used to time human developmental events during pregnancy and after birth. These include age before or from the time of birth, gestational age, menstrual age, ovulation age and fertilisation age (FA). The latter is in common use by developmental biologist and biomedical scientists. FA was chosen as it offers a relatively accurate starting reference point and can be used to define events and development windows on a single axis that can be used to compare easily the timing of important events in human development that occur both during pregnancy and after birth whether that birth is premature, at normal term or post-mature.
- 2) Definitions by different bodies have varied in their application of the term Infant. The one for EU regulatory purposes which classes infants as in the age range from the time of normal term birth to 1 year of age was used.
- 3) The term exclusive breastfeeding (EBF) indicates that the infant receives breast milk (including expressed breast milk or breast milk from a wet nurse) and allows the infant to receive oral rehydration solution, drops, syrups (vitamins, minerals, medicines), but nothing else.
- 4) EBF is recommended by WHO and many EU member states. It should be carried out from birth to 6 months of age (270–450 days FA) but not after 500 days FA.
- 5) There are several definitions of the perinatal period: in this guidance the definition from WHO 'The perinatal period commences at 22 completed weeks of gestation and ends 7 completed days after birth', i.e. 155–280 days FA is endorsed.
- 6) The usage by WHO in employing the term moderate preterm (210–245 days FA) rather than the general term preterm will be adhered to. This is in contrast to very preterm (180–210 days FA) and extremely preterm (140–180 days FA).

Appendix C – Decision tree approach to address the difference in toxicokinetics between infants below 16 weeks of age and older population for the risk assessment of substances not intentionally added to food for infants below 16 weeks of age

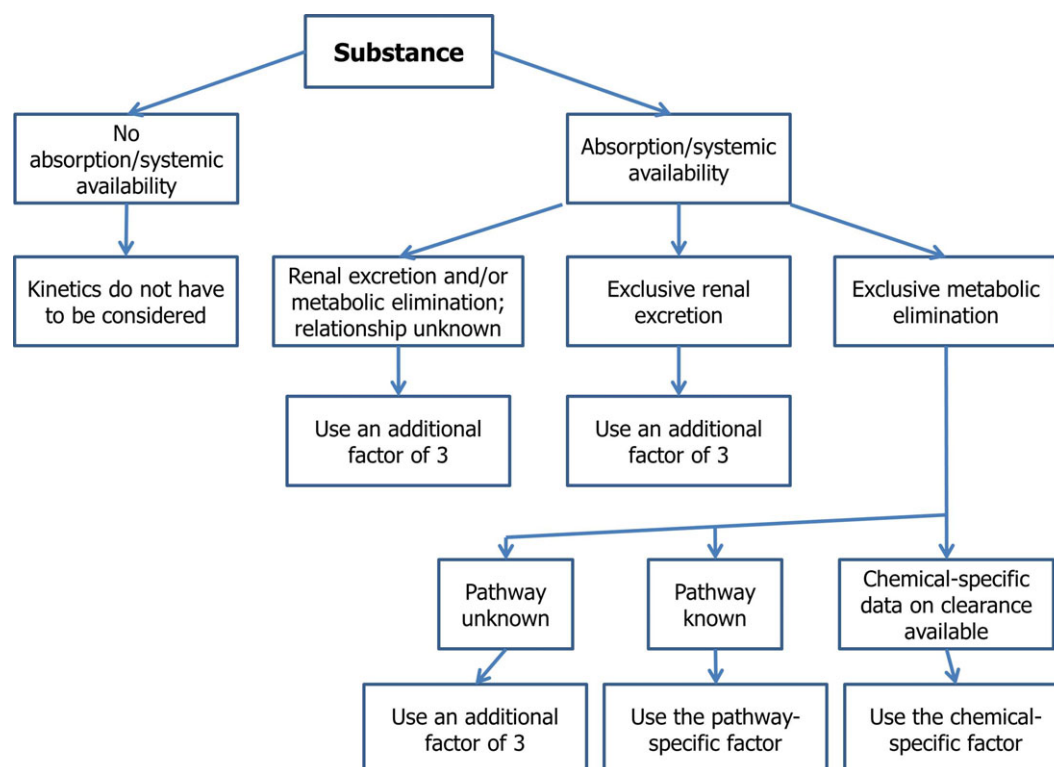


Figure C.1: The above decision tree applies exclusively to the risk assessment of substances **not** intentionally added to food for infants below 16 weeks of age. A default toxicokinetic factor of 3.2 is typically used to account for the inter-individual human variability within the general population. As discussed in Section 5, the differences between the adult population and the infants, in particular the very young ones, are in many cases greater than the default factor of 3.2 and hence necessitates an additional factor of 3 in the case where no data are available on the excretion or metabolism of a substance not intentionally added to food. In such case, an aggregate human UF of 30 needs to be applied to account for the increased inter-individual toxicokinetic variability (3×3.2 for TK and 3.2 for TD) that includes infants below 16 weeks of age. In case the pathway of metabolism is known, pathway-specific or chemical-specific factors should be added to the default factor of 3.2 to account for the increased inter-individual toxicokinetic variability between the adult population and infants below 16 weeks of age. Case-by-case consideration should be given whether the toxicodynamic sub-factor of 3.2 needs to be adjusted to the situation of the physiological development of infants below the age of 16 weeks